Netherlands organization for applied scientific research

TNO Division of Technology for Society

5864-EN-01

(2)

Report no.: R 88/488

Authors: S.H. Kay
M.C.Th. Scholten
C.T. Bowmer

MOBILITY OF SOIL CONTAMINANTS IN AN ECOSYSTEM OF TREES GROWING ON DREDGED MATERIAL - THE BROEKPOLDER (ROTTERDAM, THE NETHERLANDS)

Final report covering the period

Netherlands organization for applied scientific research

TNO-report

TNO Division of Technology for Society

P.O. Box 217
2600 AE Delft
Schoemakerstraat 97
2628 VK Delft, The Netherlands

Telex 38071 zptno nl
Phone +31 15 69 69 00

Report no.: R 88/488

Authors: S.H. Kay
M.C.Th. Scholten
C.T. Bowmer

MOBILITY OF SOIL CONTAMINANTS IN AN ECOSYSTEM OF TREES GROWING ON DREDGED MATERIAL - THE BROEKPOLDER (ROTTERDAM, THE NETHERLANDS)

Final report covering the period
April 1, 1988 - January 30, 1989

Date : 1988-12-27

Order no. : 17498

UNITED STATES ARMY

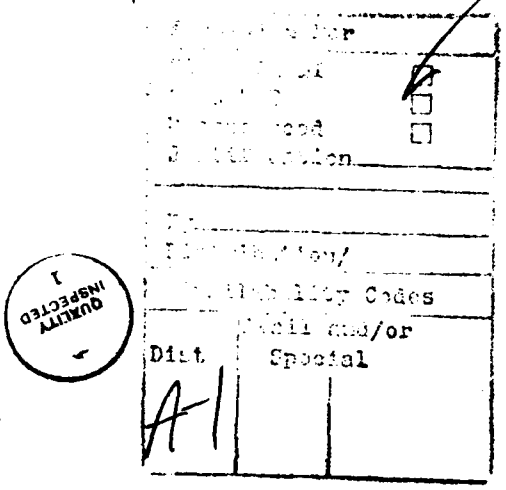
EUROPEAN RESEARCH OFFICE OF THE U.S. ARMY
London, England

Contract no.: DAJA45-87-C-0054

Approved for Public Release;
distribution unlimited.

Sponsor:

U.S. Army
Waterways Experiment Station
P.O. Box 631
VICKSBURG, MS 39180-0631
U.S.A.



CONTENTS

		Page
1.	INTRODUCTION	3
	1.1 Background	3
	1.2 Objectives	5
2.	SITE DESCRIPTION AND HISTORY	7
3.	MATERIALS AND METHODS	10
	3.1 Preliminary site survey	10
	3.2 Field collections	
	3.2.1 Soils	10
	3.2.2 Native earthworms, other invertebrates, and grasses	11
	3.2.3 Leaf litter and leaffall	12
	3.3 Plant bioassays	12
	3.4 Earthworm bioassays	14
	3.5 Combined plant and earthworm bioassay	16
	3.6 Analytical methods	16
	3.6.1 Metal analysis	16
	3.6.2 Organochlorine contaminants	17
	3.7 Statistical methods	17
	3.8 Modelling	18
4.	RESULTS AND DISCUSSION	19
	4.1 Field studies	19
	4.1.1 Characteristics of the soil profile	19
	4.1.2 Leaf litter accumulation	20
	4.1.3 Earthworm and other soil invertebrates	22
	4.2 Soils	23
	4.2.1 Physical characteristics	23
	4.2.2 Metals in soils	25
	4.2.3 Organochlorine contaminants in soils	27
	4.3 Plant bioassays and field-collected plant materials	30
	4.3.1 Plant growth characteristics	30
	4.3.2 Metals in bioassay plants, litter, and leaffall	32
	4.3.2.1 Bioaccumulation of metals in the plant bioassay	32
	4.3.2.2 Metals in litter and leaffall	35
	4.4 Earthworm bioassay and field-collected earthworms	37
	4.4.1 Growth and recovery of earthworms in the bioassay	37
	4.4.2 Metals in bioassay and field-collected earthworms	40
	4.4.3 Organochlorine contaminants in bioassay and field-collected earthworms	43
	4.5 Combined plant and earthworm bioassay	47
	4.5.1 Earthworm and plant growth response	47
	4.5.2 Bioaccumulation of contaminants in earthworms and plants	50
	4.6 Modelling	54
5.	SUMMARY AND CONCLUSIONS	57
	5.1 Summary	57
	5.2 Conclusions	59
6.	RECOMMENDATIONS	61
7.	REFERENCES	62

1. INTRODUCTION

1.1 BACKGROUND

The extensive amounts of sedimentation within navigable waters and their associated contaminant burden due to land drainage and uncontrolled waste disposal have led to concern over the fate of these contaminants in relation to the conditions of sediment disposal and the minimization of environmental effects. The need for a mechanism to assess the availability of contaminants under varying disposal alternatives has resulted in the development of plant and animal bioassay procedures.

Indeed, the Clean Water Act in the United Sates specifies that bioassays shall be conducted on contaminated sediments to predict potential toxicity, bioaccumulation, and the environmental impact of disposal prior to the initiation of dredging operations. The results of these bioassays are used to determine which disposal alternative (upland, wetland creation, or open water) has the lowest potential to cause unacceptable adverse environmental effects and what restrictions (e.g., capping) may be required for a specific disposal alternative.

The U.S. Army Engineer Waterways Experiment Station (WES) developed plant (Folsom et al., 1981a,b) and animal bioassay procedures to assess the mobility of contaminants into the food chain in confined upland and wetland-creation disposal sites. These procedures have been used successfully to predict the effects of sediment-bound contaminants upon plants and animals immediately following disposal of dredged material. Such bioassays adequately predict changes in bioavailability of certain contaminants (such as metals) which occur in upland disposal sites as the dredged material dries and changes from a reduced to an oxidized condition.

Recent evidence suggests that contaminant bioavailability in upland disposal sites changes drammatically as a terrestrial ecosystem develops and evolves toward climax conditions. These changes occur partly as the result of biological processes, such as plant growth and leaf litter accumulation. Studies at the Times Beach confined disposal site in Buffalo, New York, suggested that metal enrichment of the topsoil was occurring as a result of plant uptake by cottonwood (poplar) trees, translocation to the leaves, and

subsequent deposition during leaffall in the autumn (Marquenie et al., 1987). Preliminary studies on the Broekpolder disposal site near Rotterdam, the Netherlands, have already indicated possible surface metal enrichment of the soils due to plant uptake and deposition with leaffall (J.M. Marquenie and S.H. Kay, paper in preparation). The Broekpolder study further showed that metal uptake and deposition was different in oaks than in poplars. At both Times Beach and the Broekpolder, cadmium was enriched in the upper mineral soil layers, in comparision with the deeper layers. The extent of cadmium enrichment at both disposal sites was considered to be much greater than could have been possible if the only source has been aerial deposition. This surface enrichment was also apparent in litter-dwelling earthworms (*Lumbricus rubellus*) living on both disposal sites.

The Broekpolder and Times Beach studies are supported by other work which has demonstrated the uptake and transport of metals into tree leaves (Denayer-De Smet, 1970; Russo and Brennan, 1979; Greszta, 1982; Heale and Ormrod, 1982; Van den Burg, 1983; Grove and Malajczuk, 1985; Hagemeyer et al., 1986) as well as deposition of metals on the forest floor via leaffall (Coughtrey et al., 1979; Heinrichs and Mayer, 1980; Grove and Malajczuk, 1985). Various studies also have shown that different tree species translocate root-absorbed metals to their leaves in widely-differing amounts (Lea et al., 1979; Leavitt et al., 1979; Greszta, 1982; Heale and Ormrod, 1982; Van den Burg, 1983). Likewise, metals are retained in the leaves and appear in the litterfall to different degrees, depending both upon the specific metal and species of trees involved (Cotrufo, 1977; Zimka et al., 1981; Stachurski and Zimka, 1982; Friedland et al., 1984; Kollingbeck, 1985). Metal accumulation may inhibit the soil microflora (McCallan et al., 1941; Maliszewska et al., 1985) which break down leaf litter and thus cause the development of an abnormally thick litter accumulation (Dixon et al., 1978; Inman and Parker, 1978). The potential result may be ecosystem collapse, or more probably alterations in long-term community succession*).

Recently, regulatory scientists have expressed considerable concern about the impact that surface enrichment may have in ecosystems developing on dredged material. The potential for contaminants to move upwards through

---

*) WES-workshop

the food chain and the possibility of ecosystem collapse or succesional changes clearly indicate that a strategy is needed for the long-term management of upland dredged material disposal sites. The currently used bioassay procedures do not adequately address the long-term adverse effects of dredged material disposal in upland disposal environments. Consequently, predictive capabilities must be extended to include those potential adverse effects which do not become apparent until the newly-evolving ecosystems have reached maturity. Long-term predictive capabilities are needed for the development of management strategies, which minimize the unacceptable adverse environmental impacts of dredged material disposal. Previous studies at Times Beach and the Broekpolder suggest that minimalization of contaminant mobility could be effected through careful vegetation management.

1.2 OBJECTIVES

The primary purpose of this study is to determine whether or not plant (*Cyperus esculentus*) and earthworm (*Eisenia foetida*) bioassays applied to sediments adequately predict the long-term environmental impacts of disposed contaminated dredged material in upland sites. The information gained from this research may be used either to validate and/or modify existing bioassay procedures or to develop new procedures so that the long-term effects may be predicted prior to commencing dredging and disposal activities. The results of this project may also provide useful information for site vegetation management in minimising the movement of contaminants into the food chain.

The specific objectives of this research are:

a. To compare the results of the standard plant and earthworm bioassays of unchanged dredged material with that of environmentally modified dredged material.

b. To assess the impact of tree growth on the mobility of contaminants at an upland disposal site.

c. To compare the results of the standard bioassay with bioaccumulation of contaminants in organisms native to the disposal site.

d. To develop a model of contaminant mobility and cycling within an ecologically mature disposal site.

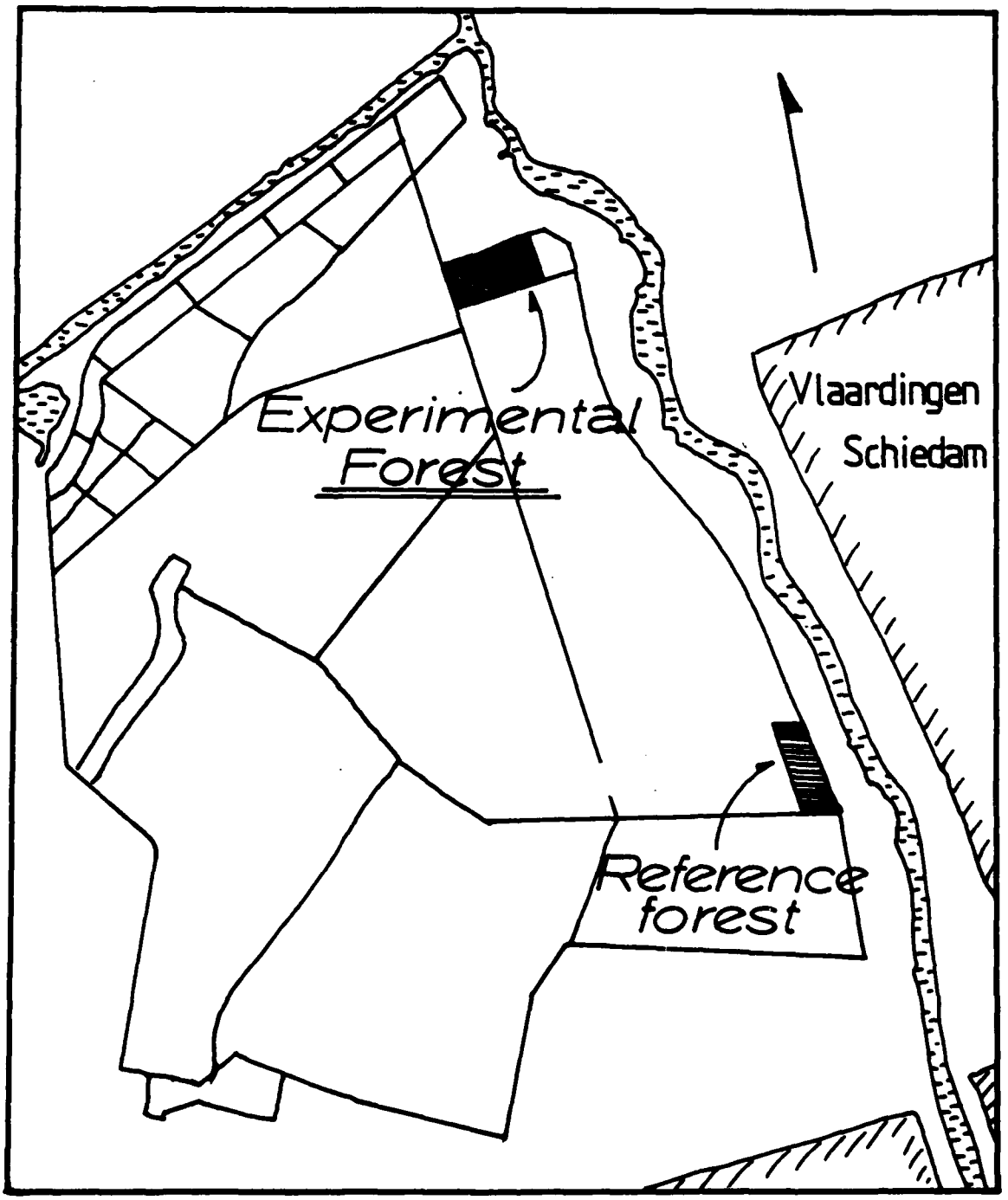
e. To estimate the sources of variances in chemical analyses of plant, animal, and substrate samples across a disposal site.

f. To assess the feasibility of combining both the plant and earthworm bioassays into a single bioassay, thereby increasing the cost effectiveness of the procedure.

2. SITE DESCRIPTION AND HISTORY

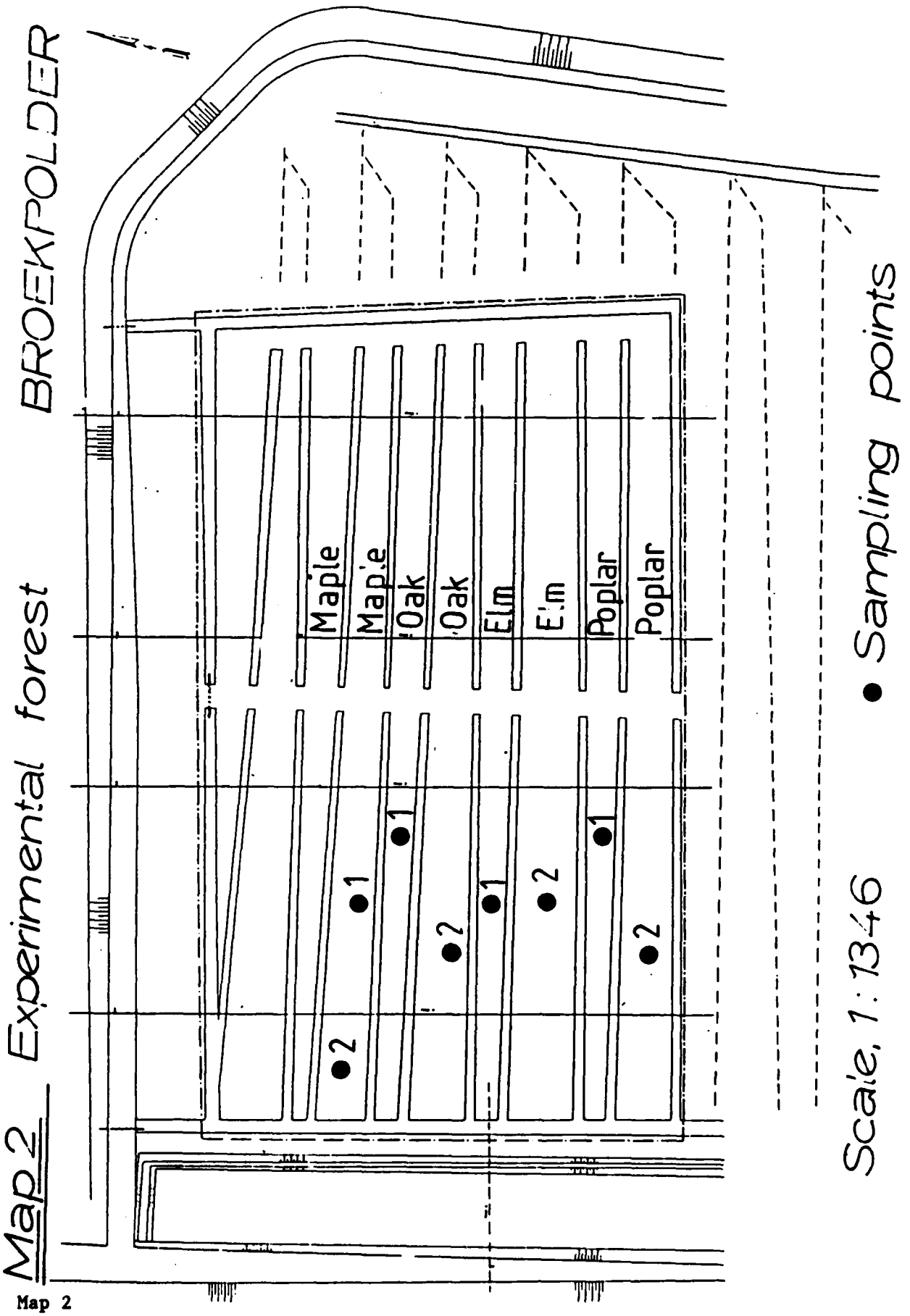
The area selected for the collection of test dredged materials is the Broekpolder which lies immediately to the N.E. of Vlaardingen, a suburb of Rotterdam (see Map 1). The Broekpolder is composed of dredged materials from the eastern Rotterdam harbour area. In this sector some 3-5 million m^3 are dredged anually and the material originates for over 90% from the river Rhine. The extreme pollution of the riverwater in former days, and recent spasmodic and regular contaminant releases from as far south-east as Switserland, has led to a severe contamination of these eastern harbour sediments. These more severely contaminated dredged materials were disposed in confirned onshore disposal sites, such as the Broekpolder. In this case a shallow lake was filled in. The Broekpolder (ca. 6 km^2) was utilised for disposal until 1967 and was filled with about 6 m of material after consolidation. Filling was done in sections with layers of ca. 1 m deep. This was followed by ripening for 3 years, rotatilling and deep dewatering before the next layer was applied, in order to enhance the leaching of marine salts. After filling was completed in 1972, one section was selected for testing the suitability of the dredged material for wood production. Five tree species ranging from valuable hardwood like oak to soft wood for the paper industry such as poplar were planted along with 13 species of shrubs for undergrowth. The tree species were planted in five oblong stands, while the shrubs were spread randomly.

Map 2 shows the layout of this experimental forest. The different tree species are separated from each other by a drainage ditch, and a further such ditch runs through the centre of each area. The western section of the experimental forest was chosen for the collection of test sediments and the fauna and flora. The location of the duplicate collection sites in the experimental forest is given in Map 2, i.e., Poplar 1, Poplar 2, Elm 1, Elm 2, etc.



Map 1 Broekpolder

Map 1


Map 2 Experimental forest BROEKPOLDER
Maple
Maple
Oak
Oak
Elm
Elm
Poplar
Poplar
1
2
Scale, 1:1346
• Sampling points

Map 2

3. MATERIALS AND METHODS

3.1 PRELIMINARY SITE SURVEY

On May 19, 1987, TNO investigators (Drs Martin Scholten and Dr Stratford Kay), accompanied by Drs Joop Marquenie (Rijkswaterstaat, the Hague), made a preliminary survey of the Broekpolder disposal site prior to initiating the field and laboratory studies. The purpose of this survey was familiarization with the site, the planning and lay out of locations for sampling and field studies, and to determine specifically where previous studies had been carried out on the basis of the similarity of understory vegetation, it was determined that the project should be confined to plots that had polar (*Poplus robustus*), oak (*Quercus robur*), maple (*Acer pseudoplatanus*), or elm (*Ulmus hollandica*) as the dominant upper-story vegetation. The specific locations for soil collection and other studies should be as uniform as possible from plot to plot, with respect to the composition of the understory vegetation. However, the distribution of the understory vegetation from plot to plot and within a single plot proved to be quite variable with respect to both density and species composition. Consequently, sampling locations were established at points where the understory vegetation was absent. This was necessary to ensure that any differences observed during the studies were the results of the influence of the tree species which dominated the different plots. Sampling locations within a plot were also sufficiently separated to allow for possible intra-site variability.

3.2 FIELD COLLECTIONS

3.2.1 Soils

On June, 1 1987, soils were collected from each of the four tree stands selected during the earlier site survey. Our initial intention was to remove soils from ten sampling points within each location using a soil auger. The auger proved to be impractical, as soils (particulary those from below the water table) could not be pulled intact to the surface. Consequently, we excavated all soils by hand, digging a pit of approximately 2 x 3 m at the center of each sampling location.

Surface litter was removed and soils were collected from the organic humus layer (0 to 5 cm depth), the oxidized mineral layer (5 to 25 cm depth), and the anaerobic mineral layer lying permanently beneath the minimum level of the water table (85 to 120 cm depth). Soils from these layers are referred to as humic, oxidized, and anaerobic, respectively, in this report. Use of these terms has no bearing on their condition during the bioassays.

The poplar, maple, and oak stands were selected for the majority of the bioassays and for all chemical analytical work, as these tree species represent early, intermediate, and late successional species, respectively. Anaerobic soils were not collected from the elm stands. All soils were transported to the laboratory in Den Helder. Mineral soils were placed in a greenhouse in 20 x 50 x 100 cm drying flats lined with polyethylene sheeting. Soils were turned daily until air dry and were then ground in a hammer mill. Humic soils were not dried or ground, in order to preserve their biological integrity. On August 13, 1987 a single humic layer soil from a nearby poplar forest elsewhere on the Broekpolder was collected. For convenience, this sample is designated as 'reference' (see Map 1). The use of the term 'reference' is not however meant to imply that the sample necessarily came from an uncontaminated area.

Subsamples of each soil were taken for the determination of pH and organic matter. A 10 g sample was moistened and stirred with 40 ml of deionized water for pH determinations. This modification of the standard WES procedure of Folsom et al. (1981 a,b) was necessary, as the humic soils completely absorbed the first 20 ml of water, rendering any measurement impossible. Samples for organic matter determination were oven dried for 48 hr at 105°C and then ashed over night at 600°C in a muffle furnace. Oven-dry weights were used to determine the weights of mineral soils to use for the plant bioassays. Ash weights were used to calculate soil organic matter as percentage loss on ignition.

3.2.2 Native earthworms, other invertebrates, and grasses

Native earthworms were collected on August 6, 7 and 13, 1987, from each location within the four treee stands and from a single location in the reference forest by extraction with 0.5% formalin solution. Following col-

lection, the worms were rinsed with deionized water and purged of their gut contents, identified to species level, counted and frozen in acid-washed, acetone-rinsed glass bottles for subsequent chemical analysis.
Snails and slugs were collected from the tree trunks and soils, respectively, from each tree stand in the experimental forest. Both animals were purged of their gut contents, washed, frozen, and archieved for possible future analyses.

The original proposal indicated that native grasses common to all tree stands would also be collected for chemical analysis. In spite of the fact that all tree stand had originally been planted with the same grasses, the preliminary site survey and all subsequent visits revealed that none were common to all plots and tree stands. Likewise, there was no evidence of other harbaceous vegetation common to all locations. Consequently, because of this total lack of comparative material this part of the work could not be carried out.

3.2.3 Leaf litter and leaffall

Leaf litter was collected in June when the soils were sampled, by removing the layer overlying the locations where soils were collected. Leaffall was collected by hand in October 1987, from each sampling location.
Leaves were hand sorted to ensure than only those from the dominant canopy species within each stand were included for chemical analysis. The leaf litter collected in June could not be sorted, however, and consequently represents a mixture of both canopy and undergrowth species. After collection, leaves sorted from the October leaffall were washed with deionized water to remove adhering soil particles. Both litter and whole leaves were dried at 70°C for 48 hr, weighed, and stored in polythylene bags at room temperature until analysis.

3.3 PLANT BIOASSAYS

A 45-day greenhouse plant bioassay of Broekpolder soils was begun on the 11th of July 1987. This bioassay followed the WES protocol (Folsom et al., 1981a,b) as closely as possible. Bain-Marie containers specified in the WES protocol were unavailable. As a substitute, plastic pots of approximately

21 cm x 23 cm ϕ, with a volume of approximately 8.5 l, with plastic plates beneath for subirrigation were used. A 2 cm polyurethane sponge was placed into the bottom of each pot and covered with a 1-cm layer of washed, fine-quartz sand to prevent soil leakage. Each soil was thoroughly mixed before filling the pots. Four kg (Dry Weight) of mineral soil from either the oxidized or anaerobic layer or 4 kg of WES reference soil was placed into each pot.

The WES reference soil was limed to pH 7 and fertilized as specified by Folsom et al. (1981a,b). Because of the substantial differences in the bulk densities in the humic soils, in comparison with the mineral soils, it was not possible to use 4 kg (ODW*) of humic soil per pot. To do so would have required containers approximately three times larger than those used for the mineral soils. Thus, the same volume of humic soil as that yielding four kg of the mineral soils was used. Soils from all three layers in the poplar, oak, and maple stands, and from the humic and oxidized layers in the elm stand were used for plant growth studies. Soils and plant materials for chemical analysis were limited to the humic and anaerobic layers from the poplar, oak, and maple stands, as specified in the original proposal.

Tubers of yellow nutsedge (*Cyperus esculentus*) were spouted in the greenhouse in moist peat moss. Unanticipated poor weather conditions and concomitant unexpected slow drying of the soils prevented the initiation of the bioassays on schedule. Consequently, the tubers sprouted and were allowed to grow until the bioassays were begun, two weeks after the original starting date. At this time, the peat moss was washed from the roots of the sprouted plants and each plant was clipped at a height of five cm above the tuber. This study was established in a completely randomized design with four replicates for the poplar, oak, and maple soils and three for the elm soils. Each experimental unit contained three plants. Randomization tables were used to assign plants to specific pots and pots to their locations on the greenhouse tables.

---

*) ODW = oven dry weight

At the outset of the study, the fresh weight of the three plants (collectively) going into each experimental unit was recorded. Three additional sets of three plants each were weighed, oven dried for 48 hr at 70°C, and reweighed. These plants were used to estimate the original dry weights of plants going into the bioassays for use in the determination of shoot relative growth rates (RGR), during the growth period. Soil moisture was monitored by the use of soil moisture tensiometers. Water was provided as required by filling plates with deionized water. At the end of the 45-day growth period, the heights of the three original plants in each pot were measured and the new shoots taller than five cm counted. All plants were then clipped at a height of five cm above the soil using a titanium scissors to prevent metal contamination, dried for 48 hr at 70°C, and weighed. Growth was reported as an above-ground biomass only. The relative growth rates for the shoots were determined. Dried plants were stored at room temperature in polythylene 'self seal' bags for later chemical analysis.

3.4 EARTHWORM BIOASSAYS

A 28-day earthworm bioassay was commenced on July 28, 17 days after the start of the plant bioassay, in order to allow a comparison between the two procedures and to synchronize both with the combined bioassay. The same humic and anaerobic soils from the poplar, oak, and maple stands as used in the plant bioassays were used for the earthworm bioassays. With the exception of the mineral soils which had been air-dried and ground, the procedure followed the most recent revision of the WES earthworm bioassay protocol.

Plexiglass cylinders 14 cm ø x 30 cm were filled to a depth of 20 cm with either soil or horse manure (as a physical control). The filled cylinders were placed on glazed clay plates and set under constant fluorescent illumination in a cold room at 15°C. Deionized water was added to the plates periodically until capillary action had moistened the soils to the surface and no more water was absorbed. Plates were kept filled with deionized water throughout the 28-day bioassay period.

Earthworms (*Eisenia sp.*) used in the bioassays, originated from cultures at the WES and TNO, Delft. Insufficient biomass of worms of suitable size was available from one source. Worms were fed on horse manure until the expo-

sures began. To facilitate handling of the worms at the end of the bioassay period, the study was established in a randomized complete block design, with one replicate (block) of each soil set up on each of four consecutive days (four replicates per soil). One manure control and one time-zero replicate also were prepared on each day. 20 g (fresh weight) of worms were weighed, counted, and placed onto the surface of each test soil after the surface had been broken up with a titanium knife. Because of possible physiological and/or behavioural differences in worms from the two different cultures, it was decided against combining all worms into a single batch for the study. There has been some uncertainty about the identity of the earthworms used in this and previous projects. Formerly both cultures were considered to be, at the most, subspecies of *Eisenia foetida*. WES and Delft worms had been classified as *E. foetida adraeae* and *E. foetida*, respectively (Bouche, 1976). For the purpose of reporting in this study, we will use the two-species classification. Therefore, the WES worms were used for replicates 1 and 2 and the Delft worms for replicates 3 and 4. A table of random numbers was used to assign worms to specific experimental units and the experimental units to their respective positions on the table in the cold room.

After 28 days, the worms were collected from the soils by hand sorting, counted, purged of their gut content for 48 hr, and frozen for storage as described previously for field-collected worms. At the end of the bioassay, the depth of burrowing in each cylinder was recorded. After the completion of all other work, the frozen tissue samples were thawed and liquefied in their storage containers using a homogenizer equipped with a titatium milling device.

Approximately 0.5 g subsamples of each homogenate were oven dried for 24 hr at 105°C, weighed, ashed in a muffle furnace for 6 hr at 600°C, and then reweighed. Organic matter was determined by placing the samples in a muffle furnace for 6 hr at 600°C, and then reweighing them. Organic matter was determined as percent loss on ignition. The remainder of the tissue homogenates were refrozen immediately and stored at 20°C until ready for chemical analysis.

3.5 COMBINED PLANT AND EARTHWORM BIOASSAY

Earthworms and plants were placed together in the same bioassay containers to assess the feasibility of combining both bioassays into one test. This combined bioassay was conducted under both plant and earthworm bioassay conditions, with the following minor modifications. Plexiglass cylinders were used in both greenhouse and coldroom tests, because worms were to be analyzed for polychlorinated biphenyl (PCB) as well as metals. Each cylinder contained a 20 cm column of anaerobic layer soil from location 2 in the poplar stand. This was equivalent to approximately 3 kg ODW, or only about 75 per cent of that used in the other plant bioassays units. Tensiometers were only used in the greenhouse portion of the combined bioassay. In the cold room, a 16-hr photoperiod was provided by two 400 W Philips SGR103 lamps suspended approximately 1 m above the cylinders. The combined bioassay was replicated four times undereach set of conditions and was conducted simultaneously with the other plant and earthworm bioassays. Plants and earthworms were handled as in their respective bioassays.

3.6 ANALYTICAL METHODS

Chemical analysis were performed on wet subsamples of homogenized earthworm tissues, oven-dried and ground plant tissues, or thoroughly-mixed air-dried soils. Data are reported on the basis of ash-free dry weight (i.e., normalized to organic matter) for earthworms and oven-dry weight for plants and soil samples.

3.6.1 Metal analysis

Soil samples

1. Standard method NEN 6465 was used for the extraction of the soil samples. Ca 2 g of the original sample was destructed for 2.5 hr with the aid of a hydrochlaric acid, sulphuric acid and Milli Q water mixture in a volume ratio of 6:2:25. After filtration through a blue band filter, the elements Zn, Cu and Cd were analysed by inductive coupled plasma-atomic emission spectrometry (ICP-AES).

2. Aditionally, 50 g samples were digested by the method of Falsom et al. (1981a,b) to estimate biologically available metals. The soils subsamples were shaken for 24 h at room temperature with a 250 ml solution of 0.005 M diethylenetriamenepentacetic acid (DTPA), 0.01 M $CaCl_2$ and 0.1 M triethanolamine. This solution was brought to pH 7.3 by the addition of HCl. Following extraction, the solutions were centrifuged at ca. 13700 g for 30 min at 4°C. The supernatant was filtered through a white band filter. The elements Cu, Zn and Cd were analysed by ICP.AES.

Analysis of leaves and tissue

Leaf samples were cut into ca. 0.5 cm^2 pieces with a Titanium scissors. Ca. 0.5 g of leaf and tissue sample was sealed by melting in a quartz ampoule and irradiated with thermal neutrons at a flux of $4x10^{12}$ $n.cm^{-2}.s^{-1}$. Following irradiation, the samples were extracted for 0.5 h with a sulphuric acid-H_2O_2 mixture. After further separation with an ion exchanger the different fractions were counted with a Sodium iodide well detector. It should be noted that leaf material was irradiated for 2 h and animal tissue for 6 h. Comparable reference materials were analysed simultaneously.

3.6.2 Organochlorine contaminants

Polychlorinated biphenyls (PCB), hexachlorobenzene (HCB), and DDE in soils and earthworms were analysed by gas chromatography using Mirex as an internal standard. Soil samples (approximately 5 g) were spiked with Mirex and subjected to soxhlet extraction. Samples were cleaned up by chromatography over alumina and dried over sodium sulfate. Earthworm tissues (1-4 g wet weight) were digested enzymatically to break down proteins and then treated similarly as for soils. Extracts were analyzed in a 50 cm fused silicia CP Sil 19CB column with electron capture detection. Recovery ranged from 95 to 105 percent. Peak identification was confirmed by mass spectrometry.
A summary of the PCB congeners analysed is presented in Table 1.

3.7 STATISTICAL METHODS

Analysis of covariance was used to determine whether or not initial plant weights had any significant influence upon either the plant growth response or metal bioaccumulation. Analysis of covariance also was used to determine

the influence of either initial worm number or average individual worm weight at time = 0 upon the final weights and numbers of worms recovered or the bioaccumulation of metals and organochlorine contaminants. Additionally, a T-test was performed to determine whether the overall response of the two worm types differed across the experiment.
Analysis of variances were used to determine the significance of differences between both soil layers, replicate sampling locations and tree stands.

3.8 MODELLING

A semi-quantative flow model, as drawn in Figure 1 (p. 51 and 52), has been calibrated on the results of the bioassays, as well as the soil, leaffall and litter analysis. The intention is to predict potential uptake of contaminants by herbs and invertebrates living in a mature ecosystem on disposal sites. The influence of various tree stands, is compared directly with the predictions from the standard bioassays results on 'fresh' dredged materials. However, the model gives a basis for further estimations of the potential dispersal of contaminants from a mature ecosystem on disposal sites to predators feeding at these sites.

Table 1 Summary of polychlorinated biphenyl (PCB) congeners analyzed.

IUPAC number	Chlorobiphenyl Congener
15	4,4'-di
28	2,4,4'-tri
44	2,2',3,5'-tetra
49	2,2',4,5'-tetra
52	2,2',5,5'-tetra
87	2,2',3,4,5'-penta
101	2,2',4,5,5'-penta
138	2,2',3,4,4',5'-hexa
153	2,2',4,4',5,5'-hexa
180	2,2',3,4,4',5,5'-hepta

4. RESULTS AND DISCUSSION

4.1 FIELD STUDIES

4.1.1 Characteristics of the soil profile

During the excavation of soils for the laboratory studies, little sign of the development of a normal soil profile was observed anywhere in the experimental forest. The transition from organic humus to the underlying mineral layer was abrupt. This distinctly contrasts to the well-developed soil profile in the poplar (i.e., cottonwood) forest at Times Beach, Buffalo, New York (Marquenie et al., 1987). Only in the oak stand was there even the slightest indication of infiltration of organic matter into the surficial mineral layer, such as would indicate the beginning of an 'Ao' horizon.

Plant roots (especially small, fibrous roots) were very abundant throughout the humic and oxidized soil layers. The larger roots were most abundant in the oxidized layer, but many also extended well into the anaerobic zone below the water table. Small fibrous roots were abundant below the water table only in the poplar stand. However, this is probably more related to the growth habit of the species than to any phenomenon peculiar to contaminated dredged material.

The depth to the water table was greater beneath the oaks than either the maples or poplars (Table 2). At location 1 in both the oak and maple stands, an oily film having a slight petroleum odour appeared in seepage water. Soils from the anaerobic zone at these two locations also contained some dark clumps which were distinctly different from the remainder of the soil and had the same petroleum odour. Soils and seepage water from other sampling locations did not contain any visible sign of petroleum-like contamination. This suggests that the anaerobic layer beneath the experimental forest is not as homogeneous in composition (horizontally) as believed previously.

4.1.2 Leaf litter accumulation

There were substantial differences in both the quality and extent of leaf litter accumulation within the different tree stands in the experimental forest (Table 2). In June, litter accumulations were thickest beneath the poplars and oaks, comparatively thin under the maples, and almost non-existent under the elms. The litter beneath the poplars and oaks was primarily well-preserved leaves, while that beneath the elms consisted primarily of newly fallen seeds and bole (trunks, branches, and twigs). The few elm leaves present were already in an advanced state of deterioration, with little except the venation remaining intact. Maple leaves were still clearly discernible but were partially deteriorated. By August, however, only the poplar stand still had a substantial accumulation of leaf litter. Poplar leaves were only partially decomposed, in contrast to complete decomposition of all leaf litter beneath the maples and elms and nearly complete decomposition beneath the oaks. Only bole litter remained under the maples and elms at this time. Quite interestingly, leaf litter was almost totally absent beneath poplars in the nearby reference forest; only bole litter remaining on the forest floor. Differences in litter decomposition rates are well documented and are due in part to the degree of lignification of the leaves (Meentemeyer, 1978; Aber and Melillo, 1982; Melillo et al., 1982; Staaf, 1987) as well as differences in C:N ratios (Howard and Howard, 1974) and phosphorus (Staaf, 1987). Oak leaves might be expected to persist longer than those of the other species, because of possibly higher leaf C:N ratios as well as greater lignification (not measured in the present study). However, Daubenmire and Prusso (1963) did not find a significant influence of lignin content on the decomposition rates of tree litter. This does not explain why the litter beneath the poplars in the experimental forest was more persistent than that beneath the oaks, even at the end of the season, whereas that in the 'reference' forest was well decomposed. These differences may be the result of differences in contaminant bioaccumulation, mobilization to the leaves, and subsequent deposition with leaf-fall, with the resultant retardation of the metabolic activity of litter-decomposing microorganisms. Metals are known to inhibit enzyme synthesis in soil microorganisms (Cole, 1977), and metal accumulation has been demonstrated to reduce the decomposition rates of forest litter (Inman and Parker, 1978) via effects on decomposing organisms (Tyler, 1972); Killham and Wainwright, 1981). It is difficult to determine precisely which contaminant(s) may be responsible, but previous work has shown strong correla-

Table 2 Qualitative characteristics of the forest floor, soils, litter, and faunal components in the experimental forest and a 'reference' poplar forest on the Broekpolder*.

Tree stand	Location	Depth to water table cm	Oily film in anaerobic layer	Petroleum odour in anaerobic soils	Mottling in anaerobic soils	Relative leaf Litter abundance June	Relative leaf Litter abundance August	Litter Integrity June	Litter Integrity August
Poplar	1	95	absent	absent	absent	thick	thick	intact	intact
	2	95	absent	absent	absent	thick	thick	intact	intact
Oak	1	120	present	distinct	slight	thick	thin	intact	MD
	2	100	absent	absent	absent	thick	thin	intact	MD
Maple	1	85	present	distinct	distinct	thin	absent	MD	absent
	2	100	absent	slight	absent	thin	absent	MD	absent
Elm	1	ND	ND	ND	ND	very thin	absent	MD**	absent
	2	ND	ND	ND	ND	very thin	absent	MD**	absent
'Reference' forest	-	ND	ND	ND	ND	ND	absent	ND	absent

* Abbreviations: ND = no data; MD = leaf litter mostly decomposed with little distinguishable other than the venation.
** Litter consisted largely of newly-fallen seeds and bole.

Table 2 Continued*.

Tree stand	Location	Roots in anaerobic layer	Roots in oxidized layer	Earthworm species and relative abundance**	Abundance of Enchytraeid worms
Poplar	1	abundant	abundant	U (85), LT (1), LC (1)	abundant
	2	abundant	abundant	U (30)	abundant
Oak	1	rare	abundant	U (47), LR (4)	common
	2	rare	abundant	U (17), LR (34)	common
Maple	1	rare	abundant	U (18), LR (31)	common
	2	rare	abundant	LR (76)	common
Elm	1	ND	ND	ND	ND
	2	ND	ND	ND	ND
'Reference' forest	-	ND	ND	LR (33), LT (5)	rare

* Abbreviations: ND = no data; U = unidentified lumbricid; LR = *Lumbricus rubellus*; LT = *Lumbricus terrestris*; LC = *Lumbricus castaneus*.
** Numbers in parentheses are the totals collected for the species at that site.

tions between Cd and Zn content of the leaf litter and the extent of litter accumulation in a forest (Coughtrey et al., 1979). Copper has also been shown to inhibit leaf litter breakdown through its influence on the activity of earthworms (Ma, 1984). The effects of contaminants (particularly metals such as lead) on soil microorganisms which contribute to the breakdown of leaf litter are well known (Maliszewska et al., 1985).

4.1.3 Earthworms and other soil invertebrates

Earthworms were abundant in the experimental forest, but the species present and their relative abundances varied widely among the tree stands (Table 2). Most significant was the presence of large numbers of a small lumbricid (unidentified) and the absence of *Lumbricus rubellus* in the poplar stands. A single specimen of *L. terrestris* was collected from the experimental forest in the poplars, but none were found in the other tree stands. All other species found in the experimental forest were shallow-burrowing, litter-dwelling forms. One other small lumbricid, *L. castaneus*, was fairly common in the poplar stand, but rare elsewhere. The dominant species in the oak and maple stands was *L. rubellus*. These were especially abundant near the bases of oak trees. The small unidentified lumbricid was also fairly abundant at one location within the oaks, but less common elsewhere. Earthworms were not collected from the elm stand, since bioassays were not conducted on elm soils and there would have been no basis for comparison. The earthworm fauna in the experimental forest was somewhat impoverished in comparison with that in the reference forest. Deep-burrowing forms were abundant in the 'reference' poplar forest, as were the various litter-dwelling species. The importance of earthworms in litter breakdown is well known (Hutson, 1980; Staaf, 1987). Consequently, the absence of the larger litter-feeding earthworms, *L. rubellus*, may be partly responsible for the litter accumulation in the experimental forest poplar stand. Another group of annelids, the *Enchytraeids*, were very abundant in humic soils from the experimental forest (especially in the poplars), but were rare in the reference forest where earthworms were collected. These data also suggest that contaminant mobilization may be influencing both the abundance and species composition of the earthworm fauna on the Broekpolder. Interestingly, Herlitzius (1987) reported that litter decomposition in woodland soils was positively correlated with the numbers of Lumbricids and negatively correlated with

numbers of Enchytraeids. This suggests that the enchytraeid population may be a good index of environmental contamination. If this is true, then the litter accumulation beneath the populars may be explained in part as the result of reduced earthworm activity due to contaminant accumulation in the litter.

Snails, *Cepaea nemoralis* (*Helix nemoralis*), and large slugs, *Arion ater*, were very abundant throughout the experimental forest on the tree trunks and on the forest floor, respectively. In the poplar stand, many snails were also present on the forest floor, but many appeared to be moribund. Brief observations in the reference forest revealed little sign of either snails or slugs, however.

4.2 SOILS

4.2.1 Physical characteristics

During the drying and grinding phase of soil preparation prior to the start of the bioassays, an occasional mottled appearance accompanied by a distinct petroleum odour was observed in some of the anaerobic layer soils (Table 2). The soils having this odour contained many hard clumps, while the oxidized and remaining anaerobic soils were brownish, crumbly in texture, and lacked any trace of petroleum odour. Soils having this mottled appearance and odour came from locations and oily appearance existed during soil collections in the experimental forest. The odour was no longer detectable after drying and grinding were complete. This petroleum odour was never observed in any of the humic soils.

Soil pH, determined on the prepared soils used in the plant bioassays, varied somewhat both within and among stands (Table 3). Humic soils ranged from slightly acidic to neutral. Those from the oxidized layers were generally the most basic, while those from the anaerobic layers ranged from neutral to slightly basic. The range of organic matter content was 36-67 percent in humic soils, 18-25 percent in oxidized soils, and 14.5-21 percent in anaerobic soils. There was no clear relationship between percent soil organic matter and soil pH.

Table 3 Percent organic matter and pH of air-dried soils from the Broekpolder experimental forest* (H = humic layer, O = oxidised layer and A = anaerobic layer).

Species	Location	Layer	n	Percent organic matter**	Soil pH
Poplar	1	H	4	67.35 a	6.53 o
	1	O	4	25.20 g	8.03 c
	1	A	4	15.03 i	7.65 f
Poplar	2	H	4	53.18 c	6.69 n
	2	O	4	19.35 h	8.07 c
	2	A	4	18.78 h	7.34 h
Oak	1	H	4	48.38 d	7.21 j
	1	O	4	17.95 hi	7.80 d
	1	A	4	19.80 h	7.24 ij
Oak	2	H	4	42.65 e	7.25 ij
	2	O	4	21.03 h	7.73 e
	2	A	4	47.23 d	7.30 hi
Maple	1	H	4	47.23 d	7.30 hi
	1	O	4	19.58 h	8.13 b
	1	A	4	21.43 h	7.58 g
Maple	2	H	4	53.05 c	7.15 k
	2	O	4	20.05 h	8.20 a
	2	A	4	14.50 i	7.76 de
Elm	1	H	3	57.33 b	6.95 m
	1	O	3	18.20 hi	8.19 a
Elm	2	H	3	36.00 f	7.02 l
	2	O	3	20.37 h	8.15 ab
WES Reference	-	-	4	2.63 j	***

* Letters in a column followed by the same letter are not significantly different at alpha = 0.05, according to Duncan's New Multiple Range procedure.

** Determined as percent weight loss on ignition.

*** Soil limed to pH 7; pH before liming = 5.69.

4.2.2 Metals in soils

Humic soils contained higher concentrations of both total and plant bioavailable (DTPA extraction) Zn and Cd than anaerobic soils throughout the experimental forest (Table 4). Bioavailable Cu was also higher in humic than anaerobic soils. Total Cu in humic soils exceeded that in anaerobic soils from the maple stand but was similar to that in the anaerobic soils elsewhere. This would suggest that Cu in Broekpolder soils is autochthonous in origin and that aerial input is a minor contributor to the Cu content of the surficial soils at this site. However, the more upwind locations (location 2 in all except the poplars) tended to have slightly higher concentrations of metals, especially Cd and Zn, than downwind locations with respect to prevailing wind direction. Interspecific differences in retention of metals and other aerially-deposited particulates have been reported and are apparently related to leaf surface texture index (Littel, 1973; Wedding et al., 1977, Dasch, 1987) and leaf area index (Dasch, 1987). This further suggests that aerial deposition was not a factor influencing the apparent enrichment of metals in the surficial soil layers. Previous reports have suggested that aerial deposition (via aerosol and particulate fallout as well as rainfall) is largely or entirely responsible for metal enrichment in surface soils (Burton and John, 1977; Heinrichs and Mayer, 1980; Friedland et al., 1984; Hogan and Wotton, 1984; Andresen et al., 1980). Studies such as these tend to minimize the influence of plant-metal bioaccumulation. Deeper mineral soil layers are usually relatively uncontaminated, in comparison with the dredged material forming the deeper mineral 'soils' of the Broekpolder and many other disposal sites. These studies have also been conducted largely in localities in which heavy aerial pollution exists, which may quite effectively mask the influence of interspecific differences in plant uptake and mobility of toxic metals. Other studies have indicated that the concentrations of metals in soils tend to be higher at the edge of a forested area and decrease toward the interior, especially moving in a downwind direction from potential sources of emissions (Dasch, 1987). Studies demonstrating gradients in metals deposition oriented along the direction of the wind have largely been in the vicinities of intense sources of aerial emissions, such as smelters (Buchauer, 1973). Backhaus and Backhaus (1987) indicated that lead deposition in remote spruce forests was higher on the exposed forest edges than in the interior; Cd distribution, however, was independent of exposure. Although the evidence is strong that, at least in most situations, metal enrichment in plants is due primarily to aerial input (Marin and

Coughtrey, 1982a), disinguishing the relative proportions of the total plant metal content which are derived from plant uptake vs aerial sources is, however, quite difficult (Martin and Coughtrey, 1982b).

Table 4 Concentrations of metals* in soils and DTPA extracts (values in parentheses are standard deviations).

Species	Location	n	DTPA Metals ($\mu g.g^{-1}$)			Total Metals ($\mu g.g^{-1}$)		
			Zn	Cu	Cd	Zn	Cu	Cd
					Humic layer			
Poplar	1	2	839 (21)	11.70 (0.14)	13.50 (0.42)	1495 (23)	102 (6)	22.20 (0.42)
	2	4	615 (27)	13.13 (0.17)	10.40 (0.47)	1475 (51)	135 (5)	20.03 (0.67)
	combined	6	690 (118)	12.65 (0.75)	11.40 (1.68)	1482 (42)	124 (18)	20.75 (1.25)
Oak	1	2	295 (21)	17.90 (2.26)	5.15 (0.35)	903 (1)	141 (4)	11.10 (0.14)
	2	2	276 (20)	21.20 (0.71)	4.65 (0.07)	1000 (32)	168 (7)	12.45 (0.64)
	combined	4	285 (20)	19.55 (2.35)	4.90 (0.36)	951 (59)	155 (16)	11.78 (0.87)
Maple	1	2	310 (52)	19.70 (2.35)	5.50 (0.60)	974 (46)	152 (4)	12.05 (0.07)
	2	2	301 (2)	18.30 (0.00)	5.50 (0.00)	859 (1)	151 (22)	11.75 (0.07)
	combined	4	305 (31)	19.00 (2.35)	5.50 (0.33)	916 (72)	151 (13)	11.90 (0.18)
Reference forest	-	1	425	25.90	6.70	925	129	11.50
					Anaerobic layer			
Poplar	1	1	97	14.30	1.50	513	84	5.30
	2	4	107 (4)	20.50 (0.52)	1.80 (0.08)	610 (10)	98 (5)	6.10 (0.29)
	combined	5	105 (6)	19.26 (2.81)	1.74 (0.15)	591 (44)	95 (8)	5.94 (0.44)
Oak	1	1	197	30.7	2.70	828	145	7.30
	2	1	96	17.4	1.90	758	119	7.70
	combined	2	147 (71)	24.05 (9.40)	2.30 (0.57)	793 (50)	132 (18)	7.50 (0.28)
Maple	1	1	186	28.50	3.10	646	111	7.10
	2	1	92	12.00	1.40	461	64	4.70
	combined	2	139 (66)	20.25 (11.67)	2.25 (1.20)	554 (131)	88 (33)	5.90 (1.70)
WES Reference	-	2	0.68 (0.03)	0.40 (0.01)	0.10 (0.00)	42 (2)	7.65 (0.07)	1.65 (0.07)

* See materials and methods for definition of DTPA and total metals.

Substantial differences in metal concentrations also existed among the three tree stands within a given soil layer in the experimental forest. This interstand variation, especially for Zn and Cd, was significantly more pronounced in the humic soil layer than in the anaerobic layer. Since the different tree stands are oriented essentially parallel to the direction of the prevailing winds, one would expect to have found similar surficial metal enrichment in all tree stands at any given distance downwind from the edge of the forest. The combination of aerosol/particulate deposition and throughfall (rain) would have been similar, regardless of tree species. Therefore, any significant differences in surficial metal enrichment would have been the result of the dominant tree species growing at that location. The data strongly support the hypothesis that the differences observed in surface metal enrichment among the tree stands on the Broekpolder are largely the result of interspecific differences in metal bioaccumulation, mobility and deposition. Such interspecific differences are known to influence the uptake and distribution of metals within forest species (Leavitt et al., 1979). An earlier study at Times Beach, in which metal enrichment occurred similarly in the surface soils of a poplar forest, also supports our contention that the surface enrichment observed on the Broekpolder is largely the result of bioaccumulation and subsequent deposition via leaffall (Marquenie et al., 1987). Data from the current study are strongly supported by the work of Stachurski and Zimka (1982) which demonstrated a 'preference for zinc' and a marked increase in 'the input of this element to soil in the form of dying plant material' among certain species, including aspen (*Populus tremuloides*). Reaves and Berrow (1979) indicated that surface Pb enrichment is most intense when the surface horizons contain high percentages of organic matter and suggested that biological cycling is a major factor contributing to this surface enrichment. Everett et al. (1986) also demonstrated surface soil enrichment for a variety of nutrients (Mn, Cu, Fe, Zn, and several non-metal nutrients) beneath the crowns of single leaf pinyon (*Pinus monophylla*) in comparison with that between trees, further supporting our hypothesis that the differences in metal enrichment of surface soils in the Broekpolder are due largely to the influence of the particular species of tree growing at a given site.

The limited data available from the 'reference' poplar forest suggest that both Zn and Cd concentrations were lower than in the surface (i.e., humic) soils from the experimental forest, while Cu concentrations may be similar. These latter data could not be compared statistically, as they were based upon a single analysis. The lowest metal concentrations measured were in the WES references soil.

4.2.3 Organochlorine contaminants in soils

In general the concentrations of organochlorine contaminants were significantly lower in the humic soils than in anaerobic soils from the same locations (Table 5). This would be expected, as most of the contaminants measured in this study were PCBs, which are relatively tightly bound to the clay fraction of soils and sediments (effectively immobilized). Furthermore these compounds are known not to be bioaccumulated and mobilized by plants to above-ground tissues (Fries and Marrow, 1981; Buckley, 1982; Bacci and Gaggi, 1985). Two notable exceptions were HCB and p,p'-DDE, both of which were more concentrated in the humic soil layers. Concentrations of the different compounds showed no obvious trend with respect to their occurrence at the two locations within a specific tree stand. The concentrations of organochlorine contaminants in the 'reference' poplar forest frequently fell between those measured in the humic and anaerobic soils in the experimental forest. However, PCB 28 occurred above detection only in the single sample from the 'reference' forest. Organochlorine concentrations were lowest in WES reference soil in nearly all cases.

Table 5 Concentrations of PCBs, HCB and DDE in soils (values in parentheses are standard deviations).

Species	Location	n	HCB	PCB-15	PCB-28	PCB-28	PCB-49	o,p'-DDE
					$\mu g.kg^{-1}$			
					Humic layer			
Poplar	1	2	73 (4)	7.10 (2.40)	<0.01 (0.00)	<0.01 (0.00)	6.50 (2.83)	2.95 (0.21)
	2	4	118 (10)	7.93 (1.05)	<0.01 (0.00)	<0.01 (0.00)	3.78 (4.58)	3.65 (2.45)
	combined	6	103 (25)	7.65 (1.41)	<0.01 (0.00)	<0.01 (0.00)	4.69 (4.02)	3.42 (1.93)
Oak	1	2	68 (11)	6.25 (0.07)	<0.01 (0.00)	<0.01 (0.00)	<0.01 (0.00)	4.15 (0.21)
	2	2	100 (11)	7.40 (0.71)	<0.01 (0.00)	<0.01 (0.00)	<0.01 (0.00)	5.45 (0.21)
	combined	4	84 (20)	6.83 (0.78)	<0.01 (0.00)	<0.01 (0.00)	<0.01 (0.00)	4.80 (0.77)
Maple	1	2	72 (20)	8.10 (0.99)	<0.01 (0.00)	<0.01 (0.00)	<0.01 (0.00)	<0.01 (0.00)
	2	2	63 (6)	5.95 (0.64)	<0.01 (0.00)	<0.01 (0.00)	<0.01 (0.00)	<0.01 (0.00)
	combined	4	67 (7)	7.03 (1.42)	<0.01 (0.00)	<0.01 (0.00)	<0.01 (0.00)	<0.01 (0.00)
Reference forest	-	1	25	17.00	12.00	3.50	7.20	<0.01
					Anaerobic layer			
Poplar	1	1	35	35.00	<0.01	3.10	8.40	<0.01
	2	4	69 (12)	62.25 (23.33)	<0.01 (0.00)	<0.01 (0.00)	13.75 (1.71)	<0.01 (0.00)
	combined	5	62 (18)	56.20 (24.31)	<0.01 (0.00)	<0.63 (1.38)	12.68 (2.81)	<0.01 (0.00)
Oak	1	1	57	63.00	<0.01	13.00	23.00	<0.01
	2	1	52	67.00	<0.01	6.00	15.00	<0.01
	combined	2	55 (4)	65.00 (2.83)	<0.01 (0.00)	9.50 (4.95)	19.00 (5.66)	<0.01 (0.00)
Maple	1	1	44	123.00	<0.01	14.00	19.00	<0.01
	2	1	33	39.00	<0.01	<0.01	7.20	<0.01
	combined	2	39 (8)	81.00 (59.40)	<0.01 (0.00)	<7.01 (9.89)	13.10 (8.34)	<0.01 (0.00)
WES Reference	-	2	8 (8)	1.55 (0.07)	<0.01 (0.00)	0.50 (0.14)	0.25 (0.07)	<0.01 (0.00)

Table 5 Continued.

Species	Location	n	PCB-101	PCB-87	p,p'-DDE	PCB-153	PCB-138	PCB-180
					$\mu g.kg^{-1}$			
					Humic layer			
Poplar	1	2	10.05 (3.17)	13.75 (17.32)	16.00 (1.41)	8.85 (0.92)	18.00 (1.41)	5.20 (0.42)
	2	4	16.00 (0.82)	1.93 (0.68)	28.25 (1.50)	12.50 (0.58)	25.25 (1.50)	7.33 (0.64)
	combined	6	14.02 (3.19)	5.87 (9.88)	22.33 (7.03)	11.28 (1.98)	22.83 (3.97)	6.62 (1.22)
Oak	1	2	10.05 (0.71)	1.75 (0.07)	11.01 (15.55)	11.00 (0.00)	25.00 (0.00)	7.30 (0.28)
	2	2	18.50 (0.71)	2.05 (0.21)	21.50 (4.95)	14.00 (1.41)	30.50 (3.54)	9.60 (1.98)
	combined	4	14.50 (4.65)	1.90 (0.22)	10.00 (11.66)	12.50 (1.91)	27.75 (3.77)	8.45 (1.76)
Maple	1	2	10.50 (0.71)	1.85 (0.21)	18.00 (2.83)	10.50 (0.71)	24.00 (1.41)	7.60 (0.42)
	2	2	11.50 (0.71)	1.75 (0.21)	17.00 (1.41)	10.20 (1.13)	24.00 (2.83)	7.45 (0.78)
	combined	4	11.00 (0.82)	1.80 (0.18)	16.25 (11.20)	10.35 (0.79)	24.00 (1.83)	7.53 (0.52)
Reference forest	-	1	7.7	2.40	4.70	16.00	29.00	10.00
					Anaerobic layer			
Poplar	1	1	19.00	2.60	7.90	27.00	34.00	13.00
	2	4	26.50 (1.29)	3.93 (0.10)	14.50 (0.58)	37.00 (2.16)	51.75 (2.87)	18.75 (0.96)
	combined	5	25.00 (3.54)	3.66 (0.60)	13.18 (2.99)	35.00 (4.85)	48.20 (8.32)	17.60 (2.70)
Oak	1	1	76.00	8.40	18.00	201.00	79.00	28.00
	2	1	28.00	4.50	13.00	41.00	58.00	21.00
	combined	2	52.00 (33.94)	6.45 (2.76)	15.50 (3.54)	121.00 (113.14)	68.50 (14.85)	24.50 (4.95)
Maple	1	1	29.00	5.40	13.00	37.00	51.00	18.00
	2	1	14.00	2.50	7.20	21.00	29.00	10.00
	combined	2	21.50 (10.61)	3.95 (2.05)	10.10 (4.10)	29.00 (11.31)	40.00 (15.56)	14.00 (5.66)
WES Reference	-	2	1.05 (0.21)	0.25 (0.07)	0.50 (0.00)	2.45 (0.49)	2.35 (0.49)	1.15 (0.35)

4.3 PLANT BIOASSAYS AND FIELD-COLLECTED PLANT MATERIALS

4.3.1 Plant growth characteristics

Analysis of covariance indicated that the initial weights of the three sprouted tubers in each experimental unit had a significant influence on the average heights of the original three plants after 45 days of growth, but had no significant effect on either the harvest dry weights (total above-ground biomass) or the number of new shoots produced in any of the soils bioassayed. However, removal of the data for the combined plant-earthworm bioassay plants from the analysis indicated that the influence of initial weight on average shoot height was only marginally significant (PR > F = 0.0551). T-tests indicated further that there were generally no substantial differences in the growth of plants on soils from the same soil layer in the two sampling locations within one tree stand.

There were significant differences in the growth of plants on soils from different tree stands and on different soil layers within a single tree stand (Table 6). The average shoot heights (of the original three plants) were consistently greater on humic and oxidized layer soils within each tree stand than on the corresponding anaerobic soils. A comparison of plants grown on different soil layers within individual tree stands shows that shoot heights were similar on humic and oxidized soils from beneath either the poplars or maples. Oxidized soils from the oak stands yielded consistently greater shoot heights than humic soils, whereas the reverse was true in the elm stands. Many of the differences were, however, only slight and not statistically significant. The shortest plants occurred on the WES reference soil. New shoot production was consistently highest on humic soils from any one tree stand and lowest on anaerobic soils from that same stand, except in the case of the oak stands, where new shoot production was similar on both oxidized and anaerobic soils. Poorest response again was on WES reference soil. Similar trends were also observed in biomass (dry weight) production and relative growth rates (RGR) of shoots, where substantially better growth occurred on humic and oxidized soils than on anaerobic soils or WES reference soil. Plant growth on Broekpolder soils was within the lower portion of the range reported previously for *Cyperus* bioassays of dredged material under upland conditions (Folsom et al., 1981a,b). This is apparently the result of day temperatures which averaged between 20 and 25°C, rather than the desired constant 30°C normally used in the

Cyperus bioassay. The differences in plant growth among soil layers within a stand or across the entire experimental forest do not appear to be related to soil pH, but more likely reflect differences in available soil nutrients (not measured).

Table 6 Plant growth response on Broekpolder soils.

Species	Location	Layer	n	Shoot height* cm	Number new shoots*	Dry weight* g	RGR*
Poplar	1	H	4	103.75 abcde	19.00 ab	11.91 fghi	1.13 bcdef
	1	O	4	105.25 abcde	18.25 abc	13.99 cdefg	1.50 a
	1	A	4	92.00 f	12.25 defg	8.14 kl	0.78 ghi
	2	H	4	106.25 abcd	20.50 ab	17.92 a	1.40 abc
	2	O	4	103.75 abcde	15.50 bcdef	13.39 defgh	1.11 cdefg
	2	A	4	97.75 cdef	11.75 defg	8.91 ijk	0.75 hi
Oak	1	H	4	104.00 abcde	18.50 abc	15.57 abcde	1.49 a
	1	O	4	112.25 a	16.00 bcde	12.88 efgh	1.46 ab
	1	A	4	94.75 ef	13.50 cdef	8.54 jkl	0.67 hij
	2	H	4	103.50 abcde	19.50 ab	14.46 bcdef	1.26 abcde
	2	O	4	107.75 abc	10.50 fgh	11.88 fghi	0.89 fghi
	2	A	4	94.75 ef	11.50 efg	10.43 hijk	0.83 fghi
Maple	1	H	4	103.25 abcdef	16.25 bcde	13.25 defgh	0.93 efghi
	1	O	4	101.00 abcdef	13.75 cdef	11.22 ghijk	0.95 efghi
	1	A	4	96.75 cdef	12.00 defg	9.03 ijk	0.90 fghi
	2	H	4	99.50 bcdef	16.75 bcd	10.64 hijk	0.78 ghi
	2	O	4	102.50 abcdef	12.50 defg	10.75 hijk	1.00 defg
	2	A	4	96.25 def	8.25 gh	8.34 jkl	0.66 ij
Elm	1	H	3	99.00 bcdef	23.00 a	17.32 ab	1.28 abcd
	1	O	3	96.33 def	18.30 abc	16.30 abcd	1.29 abcd
	2	H	3	110.33 ab	20.30 ab	16.48 abc	1.25 abcde
	2	O	3	108.00 abc	11.67 defg	11.47 fghij	1.29 abcd
WES Reference	-	-	4	82.25 g	6.00 h	5.67 l	0.38 j

* Values in a column followed by the same letter are not significantly different at alpha = 0.05, according to Duncan's New Multiple Range Test.

4.3.2 Metals in bioassay plants, litter, and leaffall

4.3.2.1 Bioaccumulation of metals in the plant bioassay

Bioaccumulation of metals by *C. esculentus* varied between soil layers from a single tree stand and within the same soil layer among different tree stands (Table 7). Variation in metal bioaccumulation between locations within a single stand generally did not appear significant, with the exception of Cd in plants on poplar humic soils and Zn in plants on oak anaerobic soils. This was most apparent in plants grown on poplar humic soils, where Cd concentrations in the bioassay plants were about twice those of plants grown on either oak or maple humic soils. There were only slight differences in Cd bioaccumulation among the plants grown on any of the anaerobic soils, however. The uptake of Cu was similar in plants grown on all soils regardless of soil layer, tree stand, or location within the stand. There were no significant differences in Zn bioaccumulation by plants grown on humic or anaerobic layer soils, except on maple soils. Between-stand differences were also minimal within a given soil layer. Concentrations of metals in plants grown on WES reference soil were generally lower than those grown on Broekpolder soils, with the exception of Cd in the anaerobic maple soils.

Linear regression analyses (Table 8) indicated significant relationships between the concentrations of both Cd or Zn in plants and those in the DTPA soil extracts, and between the concentrations of both Cu and Zn in the plants and the total concentrations of these metals in the soil. However, the low R-square valued indicated a very poor fit of the data to the linear model, that did not account for most of the variation.

Table 7 Concentrations of metals ($\mu g.g^{-1}$ dry weight) in *Cyperus esculentus* shoots after 45 days of growth on Broekpolder soils and WES reference soil.

Species	Location	Layer	n	Concentration, $\mu g.g^{-1}$* cadmium	copper	zinc
Poplar	1	H	2	11.20 (0.21)	12.43 (0.53)	214.5 (23.3)
	1	A	1	1.78	16.20	201.0
	2	H	4	5.64 (0.29)	12.32 (0.29)	202.3 (16.5)
	2	A	4	1.97 (0.26)	18.20 (4.21)	179.5 (49.3)
(combined)	1+2	H	6	7.49 (2.88)	12.36 (0.33)	206.4 (17.7)
(combined)	1+2	A	5	1.93 (0.24)	17.80 (3.75)	183.8 (43.7)
Oak	1	H	2	3.23 (0.39	11.20 (0.92)	153.0 (18.4)
	1	A	1	1.87	11.05	119.0
	2	H	2	3.80 (0.15)	12.05 (0.14)	149.0 (41.0)
	2	A	1	2.33	14.88	200.8
(combined)	1+2	H	4	3.51 (0.41)	11.63 (0.73)	151.0 (26.1)
(combined)	1+2	A	2	2.10 (0.33)	12.97 (2.71)	159.9 (57.8)
Maple	1	H	2	3.00 (0.14)	11.45 (0.92)	139.8 (3.2)
	1	A	1	1.37	14.30	205.0
	2	H	2	3.63 (0.74)	12.11 (2.40)	149.3 (3.9)
	2	A	1	1.00	15.60	197.0
(combined)	1+2	H	4	3.32 (0.57)	11.78 (1.53)	144.5 (6.2)
(combined)	1+2	A	2	1.19 (0.26)	14.95 (0.92)	201.0 (5.7)
WES Reference	-	-	2	1.47 (0.13)	8.08 (1.73)	36.6 (8.6)

* Means +/- (standard deviation); H = humic layer, A = anaerobic layer

Table 8 Linear regression analysis of metals in bioassay plants on total soil metals and DTPA extract metals.

Metal	Parameter Slope	Intercept	R-square	P > F*	CV
		Total Soil Metals**			
Cadmium	-0.0387	4.9713	0.0216	0.4829	74.4
Copper	0.0115	8.7971	0.2955	0.0050	21.6
Zinc	0.0314	82.2698	0.2201	0.0180	28.4
		DTPA Extract Metals			
Cadmium	0.6119	0.5230	0.8487	0.0001	29.2
Copper	0.1737	10.2600	0.1307	0.0758	24.0
Zinc	0.0988	135.3316	0.2199	0.0180	28.4

* Level of significance computed by the statistical packet SAS in the General Linear Models procedure, analysis of variance.

** Soil data are expressed as concentrations on the basis of ash-free dry weight (i.e., normalized to organic content).

The bioaccumulation of essential microelements is usually well regulated by most plants, whereas the uptake of nonessential elements (especially toxic metals) may be poorly regulated. In most plant Zn is stored in vacuoles, whereas Cu is stored in vacuoles or immobilized by metallothioneïne binding. These data from the present study indicate that Cu accumulation is well regulated by the plants; Cu concentrations were similar to those found in *Cyperus* spp. in freshwater marshes (Simmers et al., 1981) but substantially higher than those reported in *C. esculentus* in a greenhouse bioassay under upland conditions (Folsom et al., 1981a). Zinc uptake is influenced more so than Cu by the concentration in the bioavailable fraction, yet is fairly well regulated by the plant.

Previous studies in either the field or greenhouse and under either upland or flooded conditions showed a similar range of Zn concentrations in *Cyperus* (Simmers et al., 1981; Folsom and Lee, 1981; Folsom et al., 1981a,b). Cadmium uptake was directly correlated with the Cd concentration in the bioavailable fraction and was not regulated by the plants. Comparisons with previous studies indicate that Cd accumulation in *Cyperus* grown on Broekpolder soils was in the same range as reported for an upland greenhouse bioassay (Folsom et al., 1981a) but substantially greater than that reported for field collected plants (Simmers et al., 1981).

The lower uptake of Cu in humic soils compared to anaerobic soils might be caused by complexation of Cu to organic substances in the humic soils. This can also explain the small difference in uptake of Zn in both soil types, in spite of the higher Zn content of the humic soil, as Zn is also known to form organic complexes. Cd does not form organic complexes, thus explaining the higher Cd uptake on humic soils. The Cd content of these soils is higher in comparison to anaerobic soils.

4.3.2.2 Metals in litter and leaffall

Accumulations of metals in field-collected leaf litter (June 1987) and leaffall (October 1987) are shown in Table 9. In all cases, metal concentrations in leaffall were lower than those in the litter from the previous season within the same tree stand. This would be expected, as microbial decomposition of the leaves would logically remove organic biomass and effectively concentrate the metals in the more decomposed litter fraction. This was also reported at Times Beach (Marquenie et al., 1987). The concentrations of Cd and Zn in both litter and leaffall were greatly elevated in the poplar stand in comparison with those in either the oaks or maples. This quite clearly accounts for the elevated concentrations of Zn and Cd in poplar humic soils in comparison with those from other tree stands. A previous study also showed that poplars bioaccumulate significantly higher concentrations of zinc than oaks and many other tree species grown on the same soils (Denaeyer-De Smet, 1970). Similarly, Parker et al. (1978) reported that poplars concentrate significantly more Cd, Zn, and other metals than many other species of trees. Jackson et al. (1978) clearly demonstrated the mobilization of Cd, Cu and Zn from contaminated soils into the leaves and other portions of maples (*Acer rubrum*). Root uptake and significant translocation of Cd to leaves of pin oak seedlings (*Quercus palustris*) has also been reported (Russo and Brennan, 1979). Furr et al. (1981) similarly reported elevated levels of Cd, Cu, Ni, Pb, and Zn in leaves, fruits, and twigs of apples grown in pots containing sludge-amended soil. Cisternas and Mignolet (1982) also reported that Pb concentrations increased from litter to humic soils and that interspecific differences in litter decomposition rates had an influence on the concentrations of metals in the humic layer. The data from the present study indicate substantial interspecific influences on the concentrations of metals in the humic soils, but more

strongly support the hypothesis that interspecific differences in bioaccumulation and mobility of metals into tree leaves, with subsequent deposition on the forest floor, are largely responsible for the enrichment of Cd and Zn in surface soils on the Broekpolder. Copper concentrations in leaf litter were similar in all tree stands. Poplar and oak leaffall contained similar Cu concentrations, but that in maple leaffall appeared to be somewhat lower. This may be a function of different Cu requirements in different species or possible differences in reabsorption of Cu during senescence. The extent to which nutrients are removed from leaves during senescence is known to vary among species (Killingbeck, 1985) and plant communities (Grubb, 1977).

Table 9 Metal concentrations ($\mu g.g^{-1}$ dry weight) in leaf litter and leaffall from the Broekpolder experimental forest in 1987.

Species	Component	Cadmium	Copper	Zinc
Poplar	litter	11.85	42.4	1593
	leaffall	8.10	14.5	1495
Oak	litter	1.80	44.5	303
	leaffall	0.51	15.4	80
Maple	litter	2.03	43.7	281
	leaffall	0.35	6.6	52

* Leaf litter was collected in June and leaffall in October; the data represent one analysis from each stand.

These findings have significant implications for the management of vegetation on upland disposal sites containing contaminated dredged material. Upland disposal sites change significantly with time. Not only do metals tend to become more bioavailable as the site dewaters and dries out (Folsom and Lee, 1981; Folsom et al., 1981a), but vegetation growing on the site also modifies the environment significantly with time. The growth of vegetation over a long period of time (years) may subtantially modify the initial conditions on the site following dredging and disposal activities. In some cases, the site may be less hazardous than initially; in other cases, the site may become more hazardous with time. This was suggested previously (Marquenie, 1986; Marquenie et al., 1986; Marquenie et al., 1987). If a dredged material contains significant concentrations of potentially-toxic

metals such as Cd, the site could be managed to prevent the growth of species, such as poplars (i.e., cottonwoods), which tend to mobilize the metals and deposit them on the soil surface, effectively moving toxic metals into the environment via the food chain. At the present time, the plant bioassay cannot adequately address these long-term changes. More information is needed to determine which forest (and other) species mobilize toxic metals from lower soil strata (i.e., mineral soil layers) to the surface. Some information is already available on comparative uptake of Cd, Cu, Pb, and Zn in soils by seedling forest trees (Kelly et al., 1979; Greszta, 1982). Zimka et al. (1981) indicated that significant interspecific differences exist in the abilities of tree species to mobilize Zn: birch (*Betula verrucosa* and *B. pubescens*), alder (*Alnus glutinosa*), and lime (*Frangula alnus*) mobilize Zn to their leaves, whereas oak (*Quercus robur*), spruce (*Picea excelsa*), and pine (*Pinus silvestris*) did not. Previous work has also indicated that significant differences exist in the partitioning of nutrients, including Zn and Cu, within different-aged trees of the same species as well as within understory shrubs in comparison with upperstory species (Grove and Malajczuk, 1985). This clearly demonstrates the necessity for longer-term experimentation and monitoring, beginning as soon as the filling of upland disposal sites is complete. The available information is not sufficiently complete for the development of management recommendations. Careful comparisons of contaminant mobility and partitioning in different forest species with that in *C. esculentus* grown on the same soils are needed to extend the predictive ability of the current WES plant bioassay to assess potential long-term effects. A report by Landin (1978) lists the types of vegetation commonly occurring on dredged material disposal sites in the United States and may prove useful as an aid in the selection of forest trees, shrubs, and other species for future studies on contaminant mobility.

4.4 EARTHWORM BIOASSAY AND FIELD-COLLECTED EARTHWORMS

4.4.1 Growth and recovery of earthworms in the bioassay

Analysis of covariance indicated that the initial worm size had a statistically significant influence upon all earthworm growth and recovery variables, except depth of burrowing and the average percent change in weight per individual worm. An overall comparison of data from locations 1 and 2 using a T-test procedure indicated that the within-stand sampling location had no substantial influence on the response of the earthworms. An addi-

tional T-test to compare the performance of WES and TNO worm cultures by soil layer indicated no significant differences, except in percent recovery (i.e., numbers recovered) of worms from anaerobic soils; the differences observed were slight and did not appear to be biologically significant.

Visual examination (qualitative only) of all soils during worm collection at the end of the bioassay period revealed little evidence of reproduction (i.e., cocoon formation and presence of newly-hatched worms) in the anaerobic layer soils. Cocoons and newly-hatched worms were, however, common in humic soils and the manure controls. Earthworm recovery (number) was excellent on all soils tested, but significant losses of weight occurred on all anaerobic soils and most humic soils (Table 10). Weight loss occurred on all soils tested except the manure control and humic soils from the poplars and one location in the maples. Percent weight loss was substantial in humic soils from the oak stand. Weight loss was clearly the result of decreased body weight (percent change in weight per worm), rather than earthworm mortality. This indicates a condition of starvation, especially in the anaerobic layer soils, which contained little organic matter. The depth of burrowing was clearly correlated with all growth and recovery variables. Earthworms consistently burrowed throughout the 20-cm soil column in the humic soils, but rarely burrowed below the 10-cm depth in anaerobic soils, but most worms in the anaerobic soils were found in clumps in the upper 5 cm of the soil column, often in small excavations and cracks in the soil lined with a mucilaginous material. This habit may be a physical avoidance response to unsuitable soil conditions (i.e., texture, moisture, etc.) and/or lack of food materials in the soil column. *Eisenia* normally live in highly organic materials and do not survive long in mineral soils. Analysis of variance indicated that percent soil organic matter had a highly signficant ($P > F = 0.0001$) influence upon the depth of burrowing and all growth and recovery variables measured. Regression analysis indicated that the influence of soil organic matter was clearly correlated to growth variables, but that the relationship was non linear. The data tended to clump into two groups representing depths of burrowing (Table 10). The reason for this nonlinearity appears to be the lack of any gradient in percent soil organic matter (i.e., there were no soils with intermediate levels of organic matter). These data suggest that both the weight losses and the behavioral effects on burrowing are functions of the amount of organic matter (i.e., availability of food) or other physical conditions, rather

than chronic or subchronic toxicity effects. This contention is supported by previous studies which have demonstrated the influence of food quantity and quality (Abbott and Parker, 1981) and soil physical conditions (Edwards and Lofty, 1977; Kirkham, 1981; Reinecke and Kriel, 1981) required for growth and reproduction. However, the possible influence of soil contaminants cannot be dismissed without further experimentation.

Table 10 Earthworm recovery on Broekpolder soils in a 28-day bioassay*.

Species	Location	Layer**	n	Weight recovered g+	Percent weight change	Number recovered	Percent recovery	Average weight/worm g+	Percent change in weight/worm	Depth of burrowing, cm
Poplar	1	H	4	25.373 a	25.25 a	86.0 a	96.23 a	0.298 a	28.00 a	20.00 a
	1	A	4	15.644 e	-22.63 e	75.5 b	90.43 a	0.208 de	-15.68 d	8.75 b
	2	H	4	20.519 bc	0.65 bc	83.0 ab	95.53 a	0.248 bcd	5.35 bc	20.00 a
	2	A	4	15.767 e	-22.10 e	76.8 ab	90.83 a	0.208 de	-13.98 d	7.50 b
	(combined) 1+2	H	8	22.946 A	12.95 A	84.5 A	95.88 A	0.273 A	16.68 A	20.00 A
	(combined) 1+2	A	8	15.706 C	-22.36 C	76.1 B	90.63 AB	0.208 C	-14.83 D	8.13 B
Oak	1	H	4	17.486 de	-14.03 de	77.3 ab	89.75 a	0.233 cde	-3.28 bcd	20.00
	1	A	4	15.281 e	-25.13 e	79.0 ab	85.88 a	0.198 e	-13.33 d	8.75 b
	2	H	4	15.561 e	-23.65 e	73.5 b	87.30 a	0.215 de	-13.68 d	20.00 a
	2	A	4	15.479 e	-23.43 e	75.8 b	89.83 a	0.210 de	-13.67 d	10.00 b
	(combined) 1+2	H	8	16.523 C	-18.84 C	75.4 B	88.53 B	0.224 BC	-8.48 CD	20.00 A
	(combined) 1+2	A	8	15.379 C	-24.28 C	77.4 B	87.85 B	0.204 C	-13.50 D	9.38 B
Maple	1	H	4	20.814 bc	2.48 bc	80.8 ab	95.78 a	0.260 bc	6.95 bc	20.00 a
	1	A	4	16.717 de	-17,65 de	81.0 ab	88.70 a	0.210 de	-7.18 cd	10.00 b
	2	H	4	18.622 cd	-7.83 cd	78.8 ab	93.83 a	0.238 bcde	-2.05 bcd	20.00 a
	2	A	4	15.152 e	-25.38 e	74.0 b	88.55 a	0.205 e	-15.43 d	8.75 b
	(combined) 1+2	H	8	19.718 B	-2.68 B	79.8 AB	94.80 AB	0.249 AB	2.45 BC	20.00 A
	(combined) 1+2	A	8	15.934 C	-21.51 C	77.5 B	88.62 B	0.208 C	-11.30 D	9.38 B
WES Reference	-	-	4	21.631 b,AB	7.38 b,AB	78.5 ab,AB	94.80 a,AB	0.275 ab,A	12.45 b,AB	20.00 a,A

* Values in a column followed by the same letter are not significantly different at alpha = 0.05, according to the Duncan's New Multiple Range procedure. Lowercase letters apply to means of data with locations separate. Capital letters apply only to means of data in which both locations were combined.
** H = humic layer, A = anaerobic layer
+ Fresh weights at day 28

Table 11 Regressions of earthworm growth and recovery variables on percent soil organic matter.

Variable	Parameter					
	R-square	r	PR > F	CV	slope	Intercept
Total weight of worms recovered	0.5423	0.7364	0.0001	13.54	0.1428	12.6916
Percent change in total weight from day 0 to day 28	0.5385	0.7338	0.0001	92.90	0.7019	-37.4109
Percent change in average weight per worm	0.4810	0.6935	0.0001	235.28	0.5984	-25.8241
Depth of burrowing	0.8089	0.8994	0.0001	17.87	0.2913	4.2591
Ash-free dry weight at	0.5615	0.7493	0.0001	4.68	0.0005	0.1442

4.4.2 Metals in bioassay and field-collected earthworms

Analysis of covariance demonstrated that the bioaccumulation of all metals during the 28-day bioassay was affected by the initial size of the worms used. Cadmium concentrations in bioassay worms were substantially higher in all humic soils than in anaerobic soils from the same locations (Table 12). This phenomenon was most pronounced in the poplar and maple soils, where the humic: anaerobic Cd concentration ratios in the worms were approximately 3 to 4 and 2.5, respectively. Copper concentrations in the worms were consistently (but not significantly) higher on the anaerobic soils. In distinct contrast to Cd, there were no differences between stands in Cu bioaccumulation on either the humic or anaerobic soil layers. Zinc concentrations in the bioassay worms averaged slightly higher (not statistically significant) in humic than in anaerobic soils, except in the maples. These data suggest that bioaccumulation of essential metals if fairly well regulated in the earthworms, whereas Cd is not regulated physiologically. Comparisons of these data with previous studies indicate that the concentrations of metals in Broekpolder soils were well below the levels demonstrated to elicit either acute or chronic toxicity effects on *Eisenia* (Hartenstein et al., 1981; Neuhauser et al., 1984; Roberts and Dorough, 1984). The concentrations of Cd, Cu, and Zn in *Eisenia* in the present study are also similar to those reported by Hartenstein et al. (1980) for *Eisenia* grown on sewage sludge for up to 28 weeks, but substantially greater than those reported from previous *Eisenia* bioassays of dredged material (Simmers

et al., 1984; Marquenie et al., 1987). This supports our earlier conclusion that the weight losses and behavioural modifications observed in the laboratory bioassay are the result of unsuitable physical conditions, especially available organic matter as a food source. The Cd concentrations in worms on the humic soils were frequently much higher than those reported elsewhere for field-collected worms living on both polluted and unpolluted natural soils; concentrations in worms on the anaerobic-layer soils usually fell within the upper ranges previously reported (Andersen, 1979; Czarnowska and Jopkiewicz, 1978; Gish and Christensen, 1973; Van Hook, 1974; Ireland, 1979; Beyer et al., 1982). The Broekpolder appears to be significantly more contaminated than either many natural soils or several disposal sites in the United States, with respect to mobility of metals (particularly Cd) into the food chain.

Table 12 Metals in bioassay earthworms after 28 days on Broekpolder soils.

Species	Location	Layer	n	Concentration, $\mu g.g^{-1}$ ash-free dry weight		
				Cadmium	Copper	Zinc
Poplar	1	H	2	41.30 (11.51)	14.62 (1.35)	155 (27)
	1	A	1	13.23	16.07	130
	2	H	4	38.21 (9.40)	16.11 (0.98)	156 (13)
	2	A	4	9.93 (3.42)	18.52 (2.03)	141 (4)
	combined 1+2	H	6	39.24 (9.06)	15.62 (1.24)	156 (16)
	combined 1+2	A	5	10.59 (3.31)	18.03 (2.07)	139 (6)
Oak	1	H	2	24.81 (8.04)	15.78 (2.42)	141 (6)
	1	A	1	10.89	26.68	140
	2	H	2	20.49 (10.47)	15.09 (2.12)	148 (13)
	2	A	1	13.00	20.01	144
	combined 1+2	H	4	22.65 (8.02)	15.44 (1.90)	144 (9)
	combined 1+2	A	2	11.94 (1.49)	23.35 (4.72)	142 (3)
Maple	1	H	2	31.41 (9.43)	17.55 (2.55)	150 (17)
	1	A	1	12.43	18.35	158
	2	H	2	33.38 (11.85)	16.31 (4.04)	152 (28)
	2	A	1	13.18	18.11	152
	combined 1+2	H	4	32.39 (8.82)	16.93 (2.85)	151 (19)
	combined 1+2	A	2	12.80 (0.53)	18.23 (0.17)	156 (4)

Metal concentrations in field-collected earthworms varied across the Broekpolder (Table 13). *Lumbricus rubellus* contained substantially higher concentrations of all metals in the 'reference' poplar forest than in either the oaks or maples from the experimental forest. No valid comparison could be made with the poplar stand in the experimental forest, as *L. rubellus* was not found at that location. Earthworms (the small, unidentified lumbricid) found in the poplar stand in the experimental forest contained higher concentrations of Cd, similar Cu, and much lower Zn than *L. rubellus* collected either in the 'reference' forest or elswhere in the experimental forest. Whether or not this reflects interspecific differences or is related to differences in bioavailability of metals in the poplar stand cannot be determined from the limited data available. Substantial interspecific differences in the accumulation of metals have been reported previously (Beyer and Cromartie, 1987). This suggests that the absence of *L. rubellus* from the poplar stand in the experimental forest may be the result of greater sensitivity of that species to toxic metals. This seems to be supported by the work of Ma (1984) who found that Cu concentrations of 100-150 $mg.kg^{-1}$ in soils significnatly suppressed cocoon production of *L. rubellus*. Ma (1982) also reported Cd and Zn enrichment in *L. rubellus* growing on contaminated soils. Copper and zinc in field-collected worms from the experimental forest were generally much lower than in bioassay worms grown on humic soils from the same tree stands. Cadmium in field-collected worms from the poplar stand was slightly lower than in bioassay worms from humic soils and about three times higher than that in bioassay worms from anaerobic soils. Worms from the 'reference' poplar forest contained approximately the same concentration of Cd as the bioassay worms from any of the anaerobic Broekpolder soil treatments. The Zn concentration in 'reference' forest earthworms appeared to be much higher and Cu much lower than in bioassay worms on any soils. Concentrations of metals in the field-collected worms from the Broekpolder fell generally within the ranges reported previously for *Eisenia* bioassays of dredged material in the United States (Simmers et al., 1984; Marquenie et al., 1987) as well as for field-collected earthworms from polluted and unpolluted soils (Andersen, 1979; Czarnowska and Jopkiewicz, 1978; Gish and Christensen, 1973; Van Hook, 1974; Ireland, 1979; Beyer et al., 1982). The major exception was the high Cd concentration found in the small, unidentified lumbricid found in the poplar stand. This suggests that this lumbricid may be similar to *Eisenia*

with respect to metal bioaccumulation. The limited data on field-collected earthworms suggest that interspecific differences in feeding habit and physiological differences among *Eisenia* and the field-collected species probably had a substantial influence on metal uptake and retention. The bioassay worms feed entirely within the soil column, ingesting soil along with food particles. The field-collected species analyzed were all litter-feeding forms (Edwards and Lofty, 1972; Laird and Kroger, 1981; Satchell, 1983) and very likely did not have the same exposure to contaminants as the bioassay species. This latter conclusion seems to be supported by a recent report which compared metal bioaccumulation in *E. foetida* with common field species on the same soils under controlled conditions (Stafford and Edwards, 1985) using the WES bioassay procedure. These researchers found frequently higher levels of metal bioaccumulation in the field species than in *Eisenia*; the relationship between metal accumulation in *Eisenia* and field species was, however, linear.

Table 13 Metals in field-collected earthworms from the experimental forest and a 'reference' poplar forest on the Broekpolder.

Species	Location	$\mu g.g^{-1}$ ash-free dry weight		
		Cadmium	Copper	Zinc
Unidentified lumbricid	poplar - 2	28.40	1.22	39.6
Lumbricus rubellus	oak - 1	3.30	1.41	109.0
Lumbricus rubellus	maple - 1	6.61	2.39	130.5
Lumbricus rubellus	'reference'	15.35	3.34	208.3

4.4.3 Organochlorine contaminants in bioassay and field-collected earthworms

Bioaccumulation of organochlorine contaminants by experimental earthworms was consistently higher on anaerobic soils than on the humic soils from the same location (Table 14). The two exceptions were HCB and p,p'-DDE, which were always higher in worms on humic soils. This would be expected, as organochlorine contaminants in soils also followed this pattern. Two compounds analyzed, o,p'-DDE and PCB 28, were consistently near or below detection limits in the worms, except for two cases, PCB 28 in worms on anaerobic soils from one location each in the oaks and maples, which may have been the result of sample contamination. The concentration of PCB 44 in worms on oak anaerobic soil from location 1 was about an order of magnitude higher

than from elsewhere and may also have been the result of sample contamination. There were no obvious patterns for bioaccumulation of organochlorine contaminants in worms either between sampling locations within a stand or within a single soil layer among stands. Although many studies have been conducted in the field and laboratory regarding DDT and its metabolites (e.g., DDE), very little literature is available with which to compare PCB bioaccumulation in earthworms. Laboratory tests (21 and 24 days exposure) with *L. terrestris* on soils contaminated with organochlorine compounds (Ebing et al., 1984) showed substantially greater bioaccumulation of two PCB congeners (PCBs 14 and 28) and p,p'-DDE but substantially lower bioaccumulation of HCB than observed in our bioassays of Broekpolder soils. PCBs (total) in field-collected earthworms from the Volgermeerpolder in Amsterdam (Prins, 1982) were within the ranges equivalent to the sum of the congeners analyzed in the present laboratory bioassays of Broekpolder soils; although concentrations varied widely with species and location. Concentrations of p,p'-DDE were significantly lower, and HCB concentrations varied from lower to higher (again depending on species and location sampled) in the Volgermeerpolder worms in comparison with the present study (Prins, 1982). Other studies (Davis, 1966; Davis and Harrison, 1966; Wheatley and Hardman, 1968; Davis and Frensch, 1969; Edwards and Jeffs, 1974; Beyer and Gish, 1980; Yadav et al., 1981; Gish and Hughes, 1982; Forsyth et al., 1983) have shown wide ranges (varying with species and location) in bioaccumulation of DDE compounds and HCB (BHC) which encompass the ranges observed in the present report. Concentrations of PCBs, HCB, and p,p'-DDE in bioassay worms from the present study were significantly lower than those reported for bioassays of soils from the Times Beach disposal site in Buffalo (Marquenie, 1984; Marquenie et al., 1987). This suggests that the Broekpolder is significantly less contaminated, at least with respect to the mobility of these organochlorine compounds into the food chain, than the disposal site at Times Beach. Various toxicity studies with earthworms suggest that the ranges of organochlorine contaminants observed in the Broekpolder soils are well below threshold levels for either acute or chronic toxicity to earthworms (Davey, 1963; Edwards et al., 1967; Cathey, 1982; Roberts and Dorough, 1984). This supports the contention of Edwards (1982) that earthworms are relatively resistant to many soil organic contaminants and provides further evidence for our contention that the weight losses in our bioassay worms were not the result of organochlorine contaminant toxicity.

Table 14 Organochlorine contaminants in bioassay earthworms after 28 days on Broekpolder soils.

Species	Location	Layer	n	µg.kg^{-1} ash-free dry weight* HCB	o,p'-DDE	p,p'-DDE	PCB-15	PCB-28	PCB-52
Poplar	1	H	2	129 (4)	≤0.06 (0)	92 (4)	13 (10)	≤0.06 (0)	12 (3)
	1	A	1	71	≤0.06	84	32	≤0.06	36
	2	H	4	178 (126)	≤0.06 (0)	204 (35)	4 (4)	≤0.06 (0)	8 (9)
	2	A	4	139 (30)	≤0.06 (0)	114 (12)	11 (12)	≤0.06 (0)	49 (20)
	combined 1+2	H	6	161 (101)	≤0.06 (0)	166 (64)	7 (7)	≤0.06 (0)	9 (8)
	combined 1+2	A	5	126 (40)	≤0.06 (0)	108 (17)	14 (14)	≤0.06 (0)	47 (19)
Oak	1	H	2	151 (17)	≤0.06 (0)	204 (8)	15 (8)	≤0.06 (0)	17 (5)
	1	A	1	93	0.07	86	0.07	66.00	61
	2	H	2	247 (41)	≤0.06 (0)	168 (6)	10 (4)	≤0.06 (0)	13 (2)
	2	A	1	110	0.07	96	31	0.07	57
	combined 1+2	H	4	199 (61)	≤0.06 (0)	186 (22)	12 (6)	≤0.06 (0)	15 (4)
	combined 1+2	A	2	101 (12)	0.07 (0)	91 (7)	16 (22)	33.00 (47)	59 (3)
Maple	1	H	2	113 (44)	≤0.06 (0)	251 (25)	2 (3)	≤0.06 (0)	8 (11)
	1	A	1	72	0.07	66	39	65.00	79
	2	H	2	139 (63)	≤0.06 (0)	139 (21)	13 (14)	≤0.06 (0)	12 (17)
	2	A	1	110	≤0.06	57	17	≤0.06	24
	combined 1+2	H	4	126 (47)	≤0.06 (0)	195 (67)	8 (10)	≤0.06 (0)	10 (12)
	combined 1+2	A	2	91 (27)	≤0.07 (0)	62 (6)	28 (16)	≤33.00 (46)	51 (39)

* Means ± (standard deviation)

Table 14 continued.

Species	Location	Layer	n	µg.kg^{-1} ash-free dry weight* PCB-49	PCB-44	PCB-101	PCB-87	PCB-153	PCB-138	PCB-180
Poplar	1	H	2	19 (27)	24 (12)	53 (24)	11 (5)	104 (41)	139 (63)	50 (37)
	1	A	1	41	258	181	29	342	478	
	2	H	4	14 (28)	53 (24)	53 (10)	9 (6)	80 (21)	142 (25)	36 (10)
	2	A	4	55 (5)	165 (48)	153 (27)	23 (4)	229 (24)	333 (41)	77 (6)
	combined 1+2	H	6	16 (25)	43 (24)	53 (13)	10 (5)	88 (27)	141 (35)	41 (19)
	combined 1+2	A	5	52 (8)	184 (59)	159 (27)	24 (5)	251 (55)	362 (74)	94 (38)
Oak	1	H	2	13 (18)	8 (12)	62 (20)	24 (18)	116 (66)	198 (90)	61 (37)
	1	A	1	86	2695	285	36	352	458	106
	2	H	2	33 (35)	65 (38)	54 (5)	12 (1)	68 (8)	143 (18)	39 (4)
	2	A	1	62	447	206	34	323	461	138
	combined 1+2	H	4	23 (25)	37 (40)	60 (19)	18 (12)	92 (47)	170 (62)	50 (25)
	combined 1+2	A	2	74 (17)	1571 (1590)	246 (56)	35 (2)	337 (20)	459 (2)	122 (22)
Maple	1	H	2	26 (37)	36 (26)	59 (7)	6 (9)	106 (23)	224 (38)	53 (16)
	1	A	1	66	105	171	24	250	295	66
	2	H	2	18 (25)	36 (17)	68 (46)	16 (12)	114 (82)	190 (117)	61 (47)
	2	A	1	34	130	78	12	143	188	47
	combined 1+2	H	4	22 (26)	36 (18)	64 (27)	11 (10)	110 (49)	207 (74)	57 (29)
	combined 1+2	A	2	50 (23)	118 (17)	124 (66)	18 (8)	196 (76)	242 (76)	57 (13)

The concentrations of organochlorine contaminants in field-collected earthworms varied somewhat among the tree stands from which the worms were collected (Table 15), but no general trend was apparent. Concentrations in field-collected worms were generally much lower than in bioassay worms on soils from the same tree stands, particularly bioassay worms on anaerobic soils. This suggests that the exposure of earthworms to soil organic contaminants is quite different under field conditions than in laboratory bioassays and, probably, reflects the differences in feeding habits of the field-collected worms in comparison with *Eisenia* in the laboratory bioassays. This also suggests that the WES earthworm bioassay effectively represents a worst-case situation, at least for the less volatile organochlorine contaminants, such as the PCBs. Because of the limited data on the native earthworms collected on the Broekpolder, a good correlative relationship between organochlorine contaminant concentrations in bioassay worms and field-collected worms could not be established. A significant database exists for DDT and its metabolites in earthworms, but relatively little information is available in the literature with which to compare PCB and HCB bioaccumulation. Field-collected worms from the Volgermeerpolder generally contained much higher concentrations of HCB and slightly lower, to similar concentrations of p,p'-DDE than field-collected worms from the Broekpolder; total PCB concentrations in Volgermeerpolder worms varied from approximately similar (based on the sum of the congeners analyzed in the Broekpolder study) to significantly greater than in field-collected worms from the Broekpolder (Prins, 1982). Field-collected worms from the Broekpolder generally contained substantially lower concentrations of PCBs than native earthworms at the Times Beach disposal site in Buffalo, New York (Marquenie, 1984; Marquenie et al., 1987), at a minespoil reclamation (using slightly contaminated dredged material) site in Ottawa, Illinois (Marquenie, 1984), and at the upland experimental site in Bridgeport prior to filling with dredged material (Marquenie, 1984). The concentrations of p,p'-DDE and HCB in field-collected Broekpolder worms fell well within the ranges reported elsewhere (Davis, 1966; Davis and Harrison, 1966; Wheatley and Hardman, 1968; Davis and Frensch, 1969; Edwards and Jeffs, 1974; Beyer and Gish, 1980; Yadav et al., 1981; Gish and Hughes, 1982; Forsyth et al., 1983). Studies such as those done previously for toxic metals (Stafford and Edwards,1985) are needed to establish good correlations between organic contaminant bioaccumulation in common field earthworm species and the laboratory species, *Eisenia foetida*. Similarly, field validation is needed to

establish the relationships between contaminant bioaccumulation in the common field species in the laboratory and on the same soils at field sites (i.e., to take into account differences in feeding habits).

Table 15 Organochlorine contaminants in field-collected earthworms from the experimental forest and a 'reference' poplar forest on the Broekpolder.

Species	Location	HCB	o,p'-DDE	p,p'-DDE	PCB-15	PCB-28
		$\mu g.g^{-1}$ ash-free dry weight				
Unidentified lumbricid	poplar - 2	75	≤0.06	30	43	28
Lumbricus rubellus	oak - 1	23	≤0.06	45	17	11
Lumbricus rubellus	maple - 1	18	≤0.06	36	8	13
Lumbricus rubellus	'reference'	17	≤0.06	24	19	14

Species	Location	PCB-52	PCB-49	PCB-44	PCB-101	PCB-87
Unidentified lumbricid	poplar - 2	18	18	37	43	12
Lumbricus rubellus	oak - 1	10	17	17	17	5
Lumbricus rubellus	maple - 1	8	6	45	15	4
Lumbricus rubellus	'reference'	12	7	63	104	6

Species	Location	PCB-153	PCB-138	PCB-180
Unidentified lumbricid	poplar - 2	92	92	28
Lumbricus rubellus	oak - 1	44	52	≤13
Lumbricus rubellus	maple - 1	33	43	≤11
Lumbricus rubellus	'reference'	49	59	16

4.5 COMBINED PLANT AND EARTHWORM BIOASSAY

4.5.1 Earthworm and plant growth response

A bioassay was conducted in both the greenhouse and cool room under plant bioassay and earthworm bioassay conditions, respectively, to assess the potential for combining the two bioassays into one. Earthworm recovery was excellent in the presence of plants under the conditions usually used for the earthworm bioassay and was not significantly different from that on the same soil (anaerobic layer soil from location 2 in the poplar stand) without

plants present (Table 16). In the greenhouse, worm recovery varied widely, with excellent survival in two of the four replicates and almost no survival in the remaining two. One replicate each of WES and Delft worms had excellent survival. Under greenhouse conditions, the weight per individual worm was depressed about 50 percent, or about twice that of worms in the same soil under standard earthworm bioassay conditions, either with or without plants. This increased weight loss appears to be the result of elevated temperatures and concomitant desiccation, combined with a lack of sufficient food. Earthworms in all combined treatments congregated around the plant roots just beneath the soil surface and rarely burrowed deeper than 3 to 4 cm, perhaps in response to increased soil aeration in the vicinity of the plant roots. Another possibility is that the plant roots may have secreted some organic substances which attracted the worms, perhaps even providing a limited food source. Burrowing was somewhat deeper in the greenhouse than in the cool room. In the greenhouse, the worms tended to congregate in the cracks in the soil produced by plant root growth. This burrowing response probably was an attempt to escape from the heat and inhibit desiccation under greenhouse conditions. Light avoidance also appears to be a factor in the greenhouse.

Table 16 Comparison of earthworm recovery under standard bioassay conditions and in the combined bioassays*.

Variable	Standard Bioassay	Combined Bioassay	
		Coolroom	Greenhouse
Total weight of worms recovered (g fresh weight at day 28)	15.797 a	14.620 a	5.161 b
Percent weight change	-22.10 a	-27.70 a	-74.33 b
Number of worms recovered	76.8 a	82.3 a	38.8 a
Percent recovery	90.83 a	90.75 a	44.88 a
Average weight per worm at day 28 (g fresh weight at day 28)	0.208 a	0.180 a	0.118 b
Percent change in weight per worm	-13.98 a	-21.25 a	-48.80 b
Depth of burrowing, cm	7.5 a	5.0 a	5.3 a

* Values in a horizontal row followed by the same letter are not significantly different at alpha = 0.05, according to Duncan's New Multiple Range procedure.

Plant growth and shoot RGR were very poor under earthworm bioassay conditions but were approximately the same under greenhouse conditions as for the plants growing in the same soil without the earthworms (Table 17). The response of plants in the greenhouse varied widely when earthworms were present, however. Few new shoots were produced in the cool room, and new shoot production was significantly depressed even under greenhouse conditions when earthworms were present. This was unexpected, since a *Cyperus* bioassay on fly ash conducted in this cool room under similar temperature and light conditions resulted in excellent plant growth (Marquenie and Crawley, 1988). This previous study did not contain earthworms, however. The poor growth observed under cool room conditions may possibly have been caused by earthworm activity around the plant roots, which may have disrupted successful root establishment, whereas under the warm greenhouse conditions plant roots were already well established when the earthworms were introduced. Under greenhouse conditions, new shoots were produced largely at the interface between the soil and the wall of the clear acrylic cylinder, in contrast to fairly even dispersal of new shoot production in the standard plant bioassay containers. This appears to be a response to light. The reduction in new shoot production observed in the combined bioassay was probably the result of the reduced soil volume in the cylinders (hence, less nutrients available and more intraspecific competition) and poorer soil aeration resulting from a relatively smaller surface area in the cylinders.

Table 17 Comparison of plant growth under standard bioassay conditions and in both combined bioassays.

Variable	Standard Bioassay	Combined Bioassay	
		Coolroom	Greenhouse
Shoot height, cm	97.75 a	24.50 b	92.00 a
Number of new shoots	11.75 a	0.50 b	8.00 a
Harvest dry weight, g	8.91 a	0.16 b	9.62 b
Relative growth rate, $g.g^{-1}.day^{-1}$	0.75 a	0.01 b	0.71 a

* Values in a horizontal row followed by the same letter are not significantly different at alpha = 0.05, according to Duncan's New Multiple Range procedure.

The mineral soil was chosen for this bioassay to simulate conditions following disposal and prior to the development of a climax vegetation which might moderate conditions adverse to the development of an earthworm population. Consequently, conditions observed in the greenhouse combined bioassay may more realistically represent summertime field conditions occurring shortly after a disposal site has dewatered, a period which would normally be dominated by early successional plant species (grasses, sedges, etc.). Successful recovery of earthworms from two of the four cylinders suggests that a combined bioassay is potentially feasible, but substantial modification of the overall procedure will be needed to make conditions more suitable for the earthworms as well as to promote more normal plant growth and distribution within the bioassay containers. Larger, possibly opaque, containers which contain the same volume of soil and maintain the same surface: volume ratio may provide better aeration and stimulate more normal plant growth. A slightly lower temperature may also yield more consistent earthworm recovery and minimize weight loss.

4.5.2 Bioaccumulation of contaminants in earthworms and plants

Plants and earthworms from the greenhouse portion of the combined bioassay study were analyzed for the same contaminants as in the separate bioassays. Neither plants nor earthworms from the coldroom portion were analyzed because of poor plant growth and the assumption that similar earthworm growth as in the standard bioassay would have resulted in similar bioaccumulation in the absence of substantial plant growth. Bioaccumulation of metals by the plants and worms in the greenhouse portion of the combined bioassay was not in general significantly different than that for the plants and worms on the same soils under their respective standard bioassay conditions (Table 18). This was expected in the plants, as conditions were approximately the same as in the separate plant bioassay, but was somewhat surprising in the case of the earthworms, as elevated temperatures would normally be expected to result in increased bioaccumulation. Decreased feeding and burrowing activities in response to higher temperatures and drier soil conditions in the greenhouse may be responsible for this phenomenon.

Table 18 Comparison of metal bioaccumulation by *C. esculentus* and *E. foetida* under their respective standard bioassay conditions and in the combined bioassay under greenhouse conditions.

Bioassay	n	$\mu g.g^{-1}$* Cadmium	Copper	Zinc
		Cyperus esculentus		
Standard plant	4	1.97 (0.26)	18.20 (4.21)	180 (49)
Combined, greenhouse	2	1.31 (0.93)	17.63 (0.53)	175 (92)
		Eisenia foetida		
Standard earthworm	4	9.93 (3.42)	18.52 (2.03)	141 (4)
Combined, greenhouse	2	11.35 (6.88)	19.97 (4.50)	131 (0)

* Dry weight basis for plants, ash-free dry weight basis for earthworms.

Bioaccumulation of HCB and PCBs 52, 49, 44 and 101 were slightly, but not significantly, lower in the combined bioassay under greenhouse conditions than in the standard earthworm bioassay. Concentrations of PCBs 15, 87 and 153 were somewhat higher (not significantly, due to substantial variation in the combined bioassay) in the combined bioassay than under standard conditions, whereas p,p'-DDE and PCBs 138 and 180 appeared to be significantly greater in worms from the combined bioassay than in those from the standard bioassay. Because of the comparatively higher volatilities of HCB, DDE, and the lower molecular-weight PCBs, as well as potentially increased metabolism and elimination rates under higher temperatures, one might have expected reduced bioaccumulation of these compounds in the greenhouse. The intermediate-molecular-weight PCBs are less volatile than the low-molecular-weight PCBs, but are more readily metabolized than high-molecular-weight congeners. Consequently, the intermediate-weight PCBs would be expected to bioaccumulate, but not to the same extent as the higher-weight PCBs such as PCBs 138, 153 and 180. The implications regarding bioaccumulation, persistence, and potential toxicity of PCBs have been reviewed previously (McKinner and Sing, 1981; McFarland et al., 1986). The wide variation in the data and somewhat unexpected bioaccumulation patterns, particularly of p,p'-DDE and PCB 15, may be the result of extremely abnormal (i.e., for *Eisenia*) temperature and moisture conditions and their resultant effects upon the behaviour of the earthworms.

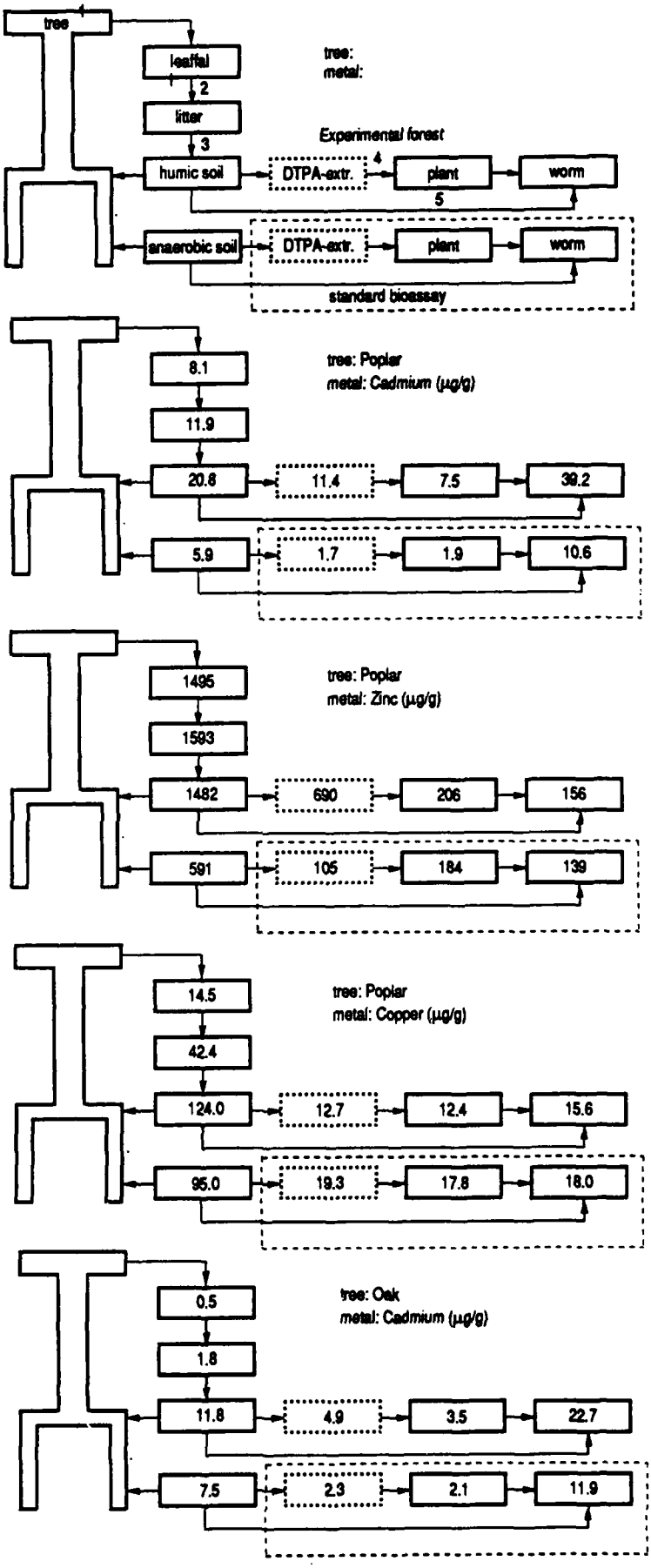


Fig. 1. The mean concentration of metals in the sequential chain: aerobic soil layer → leaffall → litter → humic soil layer (total and DTPA extractable) → plant (*Cyperus*) and worm (*Eisenia*).

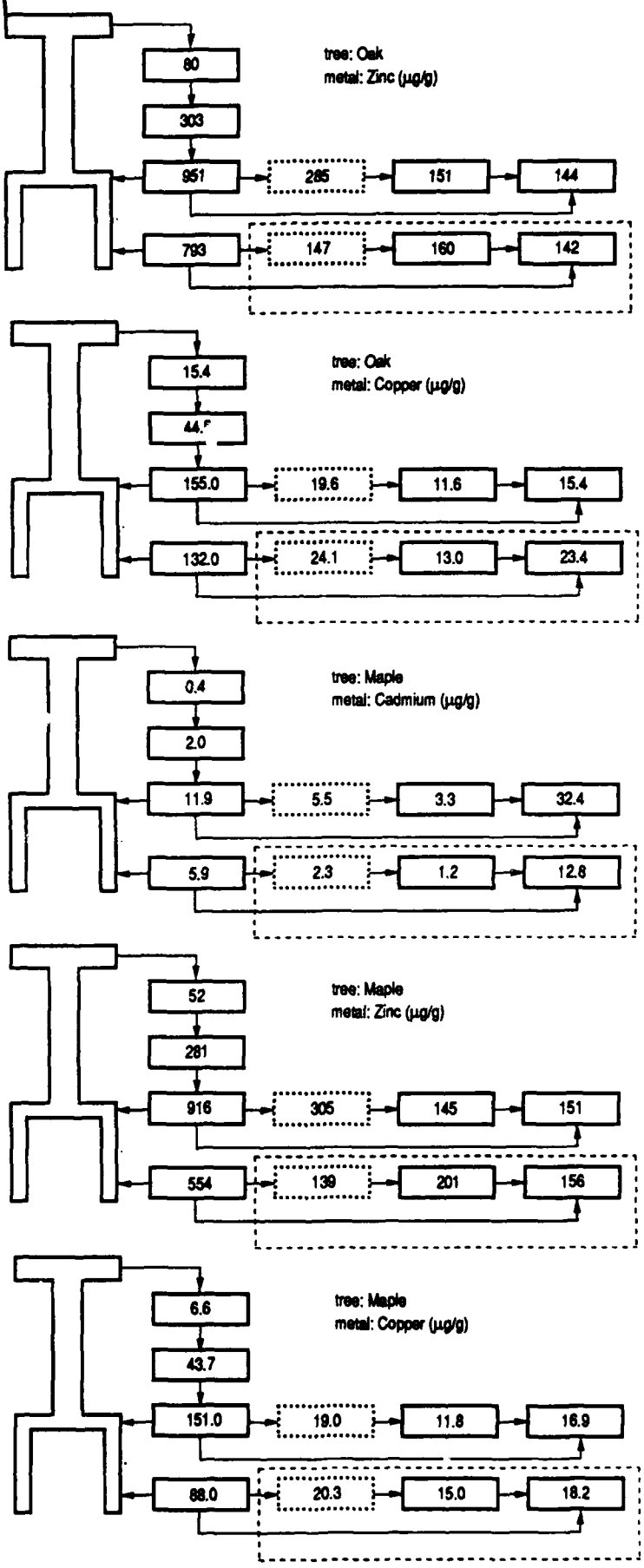


Fig. 1. Continued.

The data on bioaccumulation also suggest that a combined bioassay is feasible at some intermediate set of conditions for both temperature and moisture. The influence of temperature on contaminant bioaccumulation in both the plants and earthworms used in the standard WES bioassays has never been determined experimentally, neither has the effect of moisture on contaminant bioaccumulation in *Eisenia* been determined experimentally. This information is needed both to better relate information gained in the laboratory bioassays to field conditions and to define an intermediate set of experimental conditions under which the two bioassays might be effectively combined. Further research in this area is justified.

Tabel 19 Comparison of organochlorine bioaccumulation by *E. foetida* under standard bioassay conditions and in the combined bioassay in the greenhouse.

Contaminant	Standard conditions ($\mu g.kg^{-1}$ ash-free dry weight) n = 4	Combined, greenhouse n = 2
HCB	139 (30)	87 (123)
o,p'-DDE	≦ 0.06	≦ 0.06
p,p'-DDE	114 (12)	155 (22)
PCB-15	11 (12)	88 (124)
PCB-28	≦ 0.06	≦ 0.06
PCB-52	49 (20)	13 (18)
PCB-49	55 (5)	38 (54)
PCB-44	165 (48)	143 (131)
PCB-101	153 (27)	152 (10)
PCB-87	23 (4)	37 (21)
PCB-153	229 (24)	1290 (1423)
PCB-138	333 (41)	453 (53)
PCB-180	77 (6)	172 (57)

4.6 MODELLING

Figure 1 shows the mean concentration of metals in the sequential chain: anaerobic soil layer → leaffall → litter → humic soil layer (total and DTPA extractable fraction) → plant (*Cyperus*) and worm (*Eisenia*). The concentrations in plant (*Cyperus*) and worm (*Eisenia*) on anaerobic soils is also shown. The latter are intended to represent standard bioassay results

on 'fresh' dredged materials. The anaerobic soil layer has changed only marginally in the intervening years, and can therefore be referred to as 'fresh' material. It should be noted that the 'anaerobic soil' was dried and oxidised in the bioassay procedure.

In Table 20 the 'flow rates' between sequential compartments were given for the various metals and tree species.

Table 20 The concentration of metals (Cd, Zn, Cu) in the sequential compartments; anaerobic soil → leaffall (1); leaffall → leaflitter (2); leaf litter → humic soil (3); anaerobic soil → humic soil (1-3); humic soil → plant (4a); DTPA extractable fraction of humic soil → plant (4b) and humic soil → worm (5); are shown for the various trees and metals.

		1	2	3	1-3	4a	4b	5
Cd	poplar	1.37	1.47	1.75	3.53	0.36	0.66	1.88
	oak	0.07	3.60	6.56	1.57	0.30	0.71	1.92
	maple	0.07	5.00	5.95	2.02	0.28	0.60	2.72
Zn	poplar	2.53	1.07	0.93	2.51	0.14	0.30	0.11
	oak	0.10	3.79	3.14	1.20	0.16	0.53	0.15
	maple	0.09	5.40	3.26	1.65	0.16	0.48	0.16
Cu	poplar	0.15	2.92	8.55	1.31	0.10	0.98	0.13
	oak	0.12	2.89	3.48	1.17	0.07	0.59	0.10
	maple	0.08	6.62	3.46	1.72	0.08	0.62	0.11

The model shows the important role of tree species in the mobilization of metals into the organic, humic soil layer, which is inhabited by a large number of herbs and invertebrates. The highest input of metals to the humic soil layer through translocation of metals into fallen leaves was found in the pioneer species *populus*. Oak and maple trees show lower translocation of metals to their leaves or stronger reabsorption from the leaves before leaffall. The concentration gradient of metals from leaves to litter and humic soil, due to decomposition processes, was strongest in oak and maple litter. However, the concentration of Cd and Zn was highest in the humic layer of a poplar tree stand, due to the strong output of these metals with the leaffall of this tree. The smallest differences between the anaerobic and humic layer were found for Cu.

Bioaccumulation factors for plants did not differ much on the various humic soils. The high mobilization of metals by poplar caused a higher metal concentration (especially Cd and to a smaller extent, Zn) in *Cyperus* plants grown on humic soils collected from the poplar stand.

The bioaccumulation factor for Cd by worms was somewhat higher in humic soils from maple stands than in soils from oak or poplar. Worm bioaccumulation factors for Cu and Zn were extremely low.

The model shows large deviations in the mobility of metals as predicted by standard worm and *Cyperus* bioassays on so-called 'fresh' dredged material (cf 'anaerobic soils'), in comparison to that in mature humic soils in well developed ecosystems this is as a consequence of the different uptake and loss of metals by the vegetation present (i.e., shrubs and trees).
Large deviations were found in the case of cadmium, especially in the poplar stands. The standard bioassay clearly underestimates the mobility of metals in mature ecosystems. Smaller deviations were found for zinc. In the case of copper, the standard bioassay seemed to overestimate the bioavailability, in mature ecosystems, probably because of a low content of organic matter in 'fresh' dredged material compared to humic soils. Copper and to some extent zinc are known to form strong organic complexes with the soil organics, making the metals less available for plants and invertebrates.

No model could be built for organic contaminants as trees appear to play no important role in their mobilization. Other factors, such as volatilization, slow oxidation or biodegradation, bioaccumulation etc. may determine the actual concentration in the humic soil layer, which is mostly low compared to the anaerobic soil layer. There is not enough information available on these processes to set up a conceptual model of contaminant flow.

The standard bioassay procedure results in a respective bioaccumulation in worms and *Cyperus* that is comparable with that on (mature) humic soils. The model presented is not intended to represent mass flow through an ecosystem, it only shows the relation between concentrations in the various parts of the system. A mass flow model can be built when additional information on productivity of trees, shrubs, herbs, invertebrates and decomposition rates is available.

The model can easily be extended with a flow model of contaminants through a food chain dependent upon herbs or invertebrates.

5. SUMMARY AND CONCLUSIONS

5.1 SUMMARY

Plant (*Cyperus esculentus*) and earthworm (*Eisenia foetida*) bioassays were conducted in the greenhouse and laboratory on soils excavated from the humic and anaerobic layers in the poplar, oak and maple stands on the Broekpolder disposal site at Vlaardingen, near Rotterdam. Additional plant bioassays, assessing only growth, were conducted on soils from the oxidized mineral layer beneath the humus from each tree stand, as well as from both humic and oxidized layers in an elm stand. A preliminary test was also conducted under plant and earthworm bioassay conditions to assess the feasibility of combining the plant and earthworm bioassays.

At the end of 45- and 28-day exposure periods, respectively, plants and earthworms were analyzed for Cd, Cu and Zn. Additionally, earthworms were analyzed for several organochlorine contaminants including HCB, DDE, and PCBs. Humic and anaerobic layer soils from the poplar, oak, and maple stands, WES reference soil, and a surface soil sample from a nearby 'reference' poplar forest elsewhere on the Broekpolder were analyzed for both the metals and organochlorine contaminants.

Leaf litter, newly-fallen leaves, and native earthworms were collected from each of the above tree stands and analyzed. Snails (*Cepaea nemoralis*) and slugs (*Arion ater*) were also collected from each stand and were archived for possible future chemical analysis.

In the spring, leaf litter was abundant and well preserved in the poplar and oak stands, largely decomposed in the maples, and almost non-existent in the elms. By August, nearly all leaf litter was totally decomposed in all tree stands, except the poplars, which still contained well-preserved leaf litter. Earthworms were fairly abundant in the oaks and maples, but less so in the poplars. Deep burrowing forms were essentially absent in the entire experimental forest. Litter dwelling species, especially *Lumbricus rubellus*, were common in the oaks and maples, but absent in the poplars. A small, unidentified lumbricid was abundant in the poplars, but less common elswhere. Earthworm fauna in the experimental forest was impoverished in comparison with that found in a site in the 'reference' poplar forest.

Soil pH ranged from slightly acidic to slightly basic. Soil organic matter content was highest in humic soils (36-67%) and lowest in anaerobic soils (14.5-21%). No relationship was found between soil pH and organic matter. Humic soils generally contained higher concentrations of total and bioavailable metals than anaerobic layer soils. Concentrations of Zn and Cd were significantly greater in humic soils from the poplars than elsewhere. Organochlorine contaminants in soils generally were significantly lower in the humic layer than in anaerobic soils. Notable exceptions were HCB and p,p'-DDE, both of which were present in higher concentrations in humic than in anaerobic soils.

C. esculentus grown on humic soils contained significantly higher concentrations of Cd than those grown on anaerobic layer soils from the same locations. On humic soils from the poplar stand, Cd bioaccumulation was about twice that of plants grown on humic soils from either the oak or maple stands. Concentrations of Zn were generally similar in plants grown on humic and anaerobic soils, but bioaccumulation was depressed in oak soils, in comparison with those from the other stands. Bioaccumulation of Cu was similar on all soils across the experimental forest.

Metal concentrations in newly-fallen leaves were lower than in leaf litter from the previous season. Concentrations of Cd and Zn were greatly elevated in litter and leaffall in the poplar stand, in comparison with those in either the oaks or maples. Concentrations of Cu in leaves and leaffall were generally similar across the experimental forest.

Earthworm recovery (numbers) was excellent in all cases, but weight loss due to decreased body weight was significant on all anaerobic layer soils and most humic soils. Depth of burrowing and all growth and recovery variables were highly correlated with percent soil organic matter. Bioaccumulation of Cd by earthworms was significantly greater on humic than anaerobic layer soils, especially Cd in worms on poplar soils. Copper and Zn uptake were not significantly different, however. Bioaccumulation of HCB and o,p'-DDE by *Eisenia* was greater on humic than anaerobic layer soils; other organic compounds (PP, DDE and PCBs) were bioaccumulated more from anaerobic layer soils, however.

The small, unidentified lumbricids in the poplar stand contained higher concentrations of Cd than other species collected from either the experimental forest or the 'reference' forest. *L. rubellus* from the 'reference' forest contained higher concentrations of all metals than the same species collected at any location in the experimental forest. Concentrations of Cu and Zn in field-collected worms from the experimental forest were substantially lower than those in bioassay worms on either humic or anaerobic soils; Cd concentrations were only slightly lower than in bioassay worms on poplar humic soils and about three times higher than bioassay worms on any anaerobic layer soils. Concentrations of organochlorine compounds were lower in field-collected worms than in bioassay worms.

Earthworm recovery in the presence of plants was excellent under conditions usually used for the earthworm bioassay, but significantly depressed under plant bioassay conditions in the greenhouse. In the greenhouse, plant growth in the presence of the earthworms was similar to that in the normal plant bioassay, except for a reduction in the numbers of new shoots produced. Growth was very poor in the presence of worms under normal earthworm bioassay conditions.

Bioaccumulation of metals by the plants and worms in the greenhouse portion of the combined bioassay generally was not significantly different than that for the plants and worms on the same soils under their respective standard bioassay conditions. No analyses were conducted on the coldroom portion of the combined bioassay due to poor growth of the plants. Bioaccumulation of p,p'-DDE and PCBs 138 and 180 in the earthworms was significantly greater under greenhouse conditions than under standard earthworm bioassay conditions. The concentrations of other organochlorine contaminants in the worms were either slightly (but not significantly higher or lower than in the standard bioassay.

5.2 CONCLUSIONS

Surface soil enrichment with metals occurred on the Broekpolder largely as the result of interspecific differences in bioaccumulation, mobilization to the leaves, and deposition via leaffall. This enrichment was most pronounced for Cd, especially in the poplar stand. Surface enrichment resulted in

greater bioaccumulation of Cd and Zn in the plants and earthworms than would have been predicted from bioassays of mineral soils or newly-dredged sediments of similar composition if placed in an upland disposal site and allowed to dry and oxidize. Previous data from the Broekpolder support our contention that the concentrations of metals in anaerobic layer soils from beneath the water table have not changed substantially since filling of the site was completed. Consequently, neither the plant nor the earthworm bioassays (as currently conceived) adequately address long-term changes which occur on an upland disposal site as the result of vegetational succession and the development of a humic soil layer covered with leaf litter. This may be of great concern when upland disposal sites contain high concentrations of toxic metals, such as Cd, which are readily mobilized by certain tree species. This study clearly demonstrates that Cd is readily mobilized by poplars and suggests that upland disposal sites where Cd is a major concern should be managed to prevent the growth of poplars (i.e., cottonwoods) to minimize the mobility of Cd into the food chain.

Bioaccumulation in earthworms of organochlorine contaminants and potentially other compounds with low volatility is greatly suppressed by the growth of upper-story forest species, deposition of leaf litter, and development of an humic horizon on top of the mineral soil. Use of upland disposal sites for forestry may effectively isolate many non-volatile contaminants such as PCBs from soil fauna, as the majority of soil fauna live in the organic layers (litter and humus). This type of management could reduce the potential for bioaccumulation and biomagnification of organic contaminants in the food chain.

Combining the two bioassays into a single simultaneous bioassay may be feasible at some intermediate set of conditions in which plant growth and earthworm survival and recovery are adequate. Further development of a combined bioassay could result in substantial saving in time, labor, and analytical costs, and a reduction in space requirements, in comparison with separate bioassays conducted in different facilities at different times. Additionally, conducting a combined bioassay could prevent problems associated with the validity of later bioassays conducted on sediments or soils which have been stored or on separately-collected soils or sediments which may not have the same physical and chemical composition as the original soils. If analysis of both plants and earthworms are not required initially, tissue samples could be archived for future analyses.

6. RECOMMENDATIONS

- Poplars (i.e., cottonwoods) should not be planted or allowed to grow on contaminated disposal sites where cadmium is a significant concern.

- Forest species which minimize deposition of metals in leaffall should be planted to prevent surface metal enrichment and consequent movement of potentially toxic metals into the food chain. Those species which also have slow leaf litter decomposition rates may be desirable on sites where non-volatile organic contaminants such as PCBs are a primary concern.

- Greenhouse studies are needed to compare bioaccumulation in *C. esculentus* with that in common forest species using soils collected from different disposal sites. Field verification is also needed to compare the differences in contaminant bioaccumulation observed in seedling or sapling trees in the greenhouse bioassays with larger trees living on field sites.

- Additional research is needed to correlate organic contaminant bioaccumulation in bioassay earthworms with that in field species. Initial comparisons must be conducted under laboratory bioassay conditions using soils collected from different disposal sites. The results of these laboratory studies must be verified by analyzing the same species living on the sites where the soils were collected for laboratory bioassays.

- Vegetational management studies are needed to further ascertain which forest and herbaceous species are desirable for planting on contaminated disposal sites to minimize mobilization of contaminants into the food chain while simultaneously utilizing the full productive potential of the sites (e.g., for timber, etc.).

- Field studies on newly-filled disposal sites should be conducted to verify long-term changes in contaminant mobility which occur as the result of natural vegetational succession. These studies should include plant and earthworm bioassays as well as analyses of soils and major components of the flora and fauna.

- Studies should be conducted to determine an intermediate set of conditions suitable for a combined plant and earthworm bioassay. These studies should address the influences of temperature and moisture conditions on the bioaccumulation of contaminants by both *C. esculentus* and *E. foetida* as well as bioassay container design.

- Analysis of the remaining plant and animal tissue samples and soils would provide a broader data base for statistical comparison and yield valuable data on other components of the food chain. Remaining samples include: unanalyzed replicates of soils, *Cyperus*, and *Eisenia* from the bioassays; additional leaf litter, leaffall, and native earthworm samples from the second location in each tree stand and 'reference' forest; soil and *Cyperus* samples from growth bioassays conducted on elm soils and oxidized layer soils from the poplar, oak, and maple stands; and slugs and snails collected from the experimental forest; these samples were archived until July 1990.

- Analysis of plants, soils, and earthworms for other toxic metals including lead, nickel, arsenic and chromium, would be desirable.

- Studies are needed to determine whether or not weight losses in bioassay earthworms as the result of starvation, significantly change patterns of contaminant bioaccumulation during laboratory bioassays.

7. REFERENCES

Abbott, I. and C.A. Parker, 1981
Interactions between earthworms and their soil environment.
Soil Biol. Biochem. 13: 191-197.

Aber, J.D. and J.M. Melillo, 1982
Nitrogen immobilization in decaying hardwood leaf litter as a function of initial nitrogen and lignin content.
Can. J. Bot., 60: 2263-2269.

Andersen, C., 1979
Cadmium, lead and calcium content, number and biomass, in earthworms (Lumbricidae) from sewage sludge treated soil.
Pedobiologia, 19: 309-319.

Andresen, A.M., A.H. Johnson and T.G. Siccama, 1980
Levels of lead, copper, and zinc in the forest floor in the northeastern United States.
J. Environ. Qual., 9: 293-296.

Bacci, E. and C. Gaggi, 1985
Polychlorinated biphenyls in plant foliage: Translocation or volatilization from contaminated soils.
Bull. Environ. Contam. Toxicol., 35: 673-681.

Backhaus, B. and R. Backhaus, 1987
Distribution of long range transported lead and cadmium in spruce stands affected by forest decline.
Sci. Total Environ., 59: 283-290.

Beyer, W.N. and C.D. Gish, 1980
Persistence in earthworms and potential hazards to birds of soil applied DDT, dieldrin and heptachlor.
J. Appl. Ecol., 17: 295-307.

Beyer, W.N. and E.J. Cromartie, 1987
A survey of Pb, Cu, Zn, Cd, Cr, As, and Se in earthworms and soil from diverse sites.
Environ. Monit. Assess, 8: 27-36.

Beyer, W.N., R.L. Chaney and B.M. Mulhern, 1982
Heavy metal concentrations in earthworms from soil amended with sewage sludge.
J. Environ. Qual., 11: 381-385.

Bouche, M.B., 1976
Contribution a la stablisation de la nomenclature des Lumbricidae, *Oligochaeta I*. Synonymies et homonymies d'especes du Bassin Parisien.
Bull. Mus. Natn. Hist. Nat (Ser. 3, No. 354) Zool., 247: 81-88.

Buchauer, M.J., 1973
Contamination of soil and vegetation near a zinc smelter by zinc, cadmium, copper, and lead.
Environ. Sci. Technol., 7: 131-135.

Buckley, E.H., 1982
Accumulation of airborne polychlorinated biphenyls in foliage.
Science, 216: 520-522.

Burg, J. van den, 1983
Copper uptake by some forest tree species from an acid sandy soil.
Plant Soil 75: 213-219.

Burton, K.W. and E. John, 1977
A study of heavy metal contamination in the Rhondda Fawr. South Wales.
Water Air Soil Pollut., 7: 45-68.

Cathey, B., 1982
Comparative toxicities of five insecticides to the earthworm, *Lumbricus terrestris*.
Agricul. Environ., 7: 73-81.

Cisternas, R. and R. Mignolet, 1982
Accumulation of lead in decomposing litter.
Oikos, 38: 361-364.

Cole, M.A., 1977
Lead inhibition of enzyme synthesis in soil.
Appl. Environ. Microbiol., 33: 262-268.

Cotrufo, C. 1977
Nutrient content in litterfall of an Appalachian hardwood stand.
J. Mitchell Society, 93: 27-33.

Coughtrey, P.J., C.H. Jones, M.H. Martin and S.W. Shales, 1979
Litter accumulation in woodlands contaminated by Pb, Zn, Cd and Cu.
Oecologia (Berl.), 39: 51-60.

Czarnowska, K. and K. Jopkiewics, 1978
Heavy metals in earthworms as an index of soil contamination.
Pol. J. Soil Sci., 11: 57-62.

Dash, J.M., 1987
Measurement of dry deposition to surfaces in deciduous and pine canopies.
Environ. Pollut., 44: 261-277.

Daubenmire, R. and D.C. Prusso, 1963
Studies of the decomposition rates of tree litter.
Ecology, 44: 589-592.

Davey, S.P., 1963
Effects of chemicals on earthworms: A review of the literature.
Special Scientific Report wildlife No. 74, U.S. Dept. of the Interior, Washington DC, 20 pp.

Davis, B.N.K., 1966
Soil animals as vectors of organochlorine insecticides for ground-feeding birds. pp. 133-139.
In N.W. Moore, ed., Pesticides in the Environment and their Effects on Wildlife, Blackwell Scientific Publications, Oxford.

Davis, B.N.K. and M.C. French, 1969
The accumulation and loss of organochlorine insecticide residues by beetles, worms and slugs in sprayed fields.
Soil Biol. Biochem., 1: 45-55.

Davis, B.N.K. and R.B. Harrison, 1966
Organochlorine insecticide residues in soil invertebrates.
Nature, 211: 1424-1425.

Denayer-De Smet, S., 1970
Considerations sur l'accumulation du zinc par les plantes poussant sur sols calaminaires.
Bull. Instr. r. Sci. nat. Belg., 46: 1-13

Dixon, K.R., R.J. Luxmoore and C.L. Begovich, 1978
CERES - A model of forest stand biomass dynamics for predicting trace contaminant, nutrient, and water effects. II Model application.
Ecol. Model., 5: 93-114.

Ebing, W., J. Pflugmacher and A. Haque, 1984
Der Regenwurm als Schlusselorganisms zur Messung der Bodenbelastung mit organischen Fremdchemikalien.
Ber. Landwirtsch., 62: 222-255.

Edwards, C.A., 1982
Problems caused by the contamination of agricultural land and woodlands by toxic chemicals.
Decheniana - Beihofte (Bonn), 26: 145-150.

Edwards, C.A., E.B. Dennis and D.W. Empson, 1967
Pesticides and the soil fauna: effects of aldrin and DDT in an arable field.
Ann. Appl. Biol., 60: 11-22.

Edwards, C.A. and K. Jeffs, 1974
Rate of uptake of DDT from soil by earthworms.
Nature, 247: 157-158.

Edwards, C.A. and J.R. Lofty, 1977
Biology of Earthworms.
Chapman and Hall, London

Everett, R., S. Sharrow and D. Thran, 1986
Soil nutrient distribution under and adjacent to single leaf pinyon crowns.
Soil Sci. Soc. Amer. J., 50: 788-792.

Folsom, B.L. Jr. and C.R. Lee, 1981
Zinc and cadmium uptake by the freshwater marsh plant *Cyperus esculentus* grown in contaminated sediments under reduced (flooded) and oxidized (upland) disposal conditions.
J. Plant Nutrition, 3: 233-244.

Folsom, B.L. Jr., C.R. Lee and D.J. Bates, 1981a
Influence of disposal environment on availability and plant uptake of heavy metals in dredged material.
Tech. Rept. EL-81-12, U.S. Army Engineer Waterways Experiment Station, Vicksbur, Miss., 158 pp.

Folsom, B.L. Jr., C.R. Lee and K.M. Preson, 1981b
Plant bioassay of materials from the Blue River dredging project.
Misc. Paper EL-81-6, U.S. Army Engineer Waterways Experiment Station, Vicksburg, Miss., 57 pp.

Forsyth, D.J., T.J. Peterle and L.W. Bandy, 1983
Persistence and transfer of 36Cl-DDT in the soil and biota of an old-field ecosystem: a six-year balance study.
Ecology, 64: 1620-1636.

Friedland, A.J., A.H. Johnson, T.G. Siccama and D.L. Mader, 1984
Trace metal profiles in the forest floor of New England.
Soil Sci. Soc. Amer. J., 48: 422-425.

Fries, G.F. and G.S. Marrow, 1981
Chlorobiphenyl movement from soil to soyabean plants.
J. Agric. Food Chem., 29: 757-759.

Furr, A.K., T.F. Parkinson, D.C. Elfving, C.A. Bache, W.H. Gutenmann, G. J. Doss and D.J. Liks, 1981
Elemental content of vegetables and apple trees grown on Syracuse sludge-amended soils.
J. Agric. Food Chem., 29: 156-160.

Gish, C.D. and R.E. Christensen, 1973
Cadmium, nickel, lead, and zinc in earthworms from roadside soil.
Environ. Sci. Technol., 11: 1060-1062.

Gish, C.D. and D.L. Hughes, 1982
Residues of DDT, dieldrin, and heptachlor in earthworms during two years following application.
Special Scientific Rept. - Wildlife No. 241, U.S. Dept. of the Interior, Fish and Wildlife Service, 15 pp.

Greszta, J., 1982
Correlation between the content of copper, zinc, lead and cadmium in the soil and the content of these metals in the seedlings of selected forest tree species.
Fragmenta Floristica et Geobotanica, 28: 29-52.

Grove, T.S. and N. Malajczuk, 1985
Nutrient accumulation by trees and understorey shrubs in an age-series of *Eucalyptus diversicolor* F. Muell. Stands.
Forest Ecol. Manage., 11: 75-95.

Grubb, P.J., 1977
Control of forest growth and distribution on wet tropical mountains: with special reference to mineral nutrition.
Ann. Rev. Ecol. Syst., 8: 83-107.

Hagemeyer, J., H. Kahle and S.-W. Breckle, 1968.
Cadmium in *Fagus sylvatica* L. trees and seedlings: leaching, uptake and interconnection with transpiration.
Water Air Soil Pollut., 29: 347-359.

Hartenstein, R., E.F. Neuhauser and J. Collier, 1980
Accumulation of heavy metals in the earthworm *Eisenia foetida*.
J. Environ. Qual., 9: 23-26.

Hartenstein, R., E.F. Neuhauser and A. Narahara, 1981
Effects of heavy metal and other elemental additives to activated sludge on growth of *Eisenia foetida*.
J. Environ. Qual., 10: 372-376.

Heale, E.L. and D.P. Ormrod, 1982
Effects of nickel and copper on *Acer rubrum, Cornus stolonifera, Lonicera tatarica*, and *Pinus resinosa*.
Can. J. Bot., 60: 2674-2681.

Heinrichs, H. and R. Mayer, 1980
The role of forest vegetation in the biogeochemical cycle of heavy metals.
J. Environ. Qual., 9: 111-118.

Herlitzius, H., 1987
Decomposition in five woodland soils: relationships with some invertebrate populations and with weather.
Biol. Fertil. Soils, 3: 85-89.

Hogan, G.D. and D.L. Wotton, 1984
Pollutant distribution and effects in forests adjacent to smelters.
J. Environ., Qual., 13: 377-382.

Howard, P.J.A. and D.M. Howard, 1974
Microbial decomposition of tree and shrub leaf litter.
1. Weight loss and chemical composition of decomposing litter.
Oikos, 25: 341-352.

Hutson, B.R., 1980
The influence on soil development of the invertebrate fauna colonizing industrial reclamation sites.
J. Appl. Ecol., 17: 277-286.

Inman, J.C. and G.R. Parker, 1978
Decomposition and heavy metal dynamics of forest litter in northwestern Indiana.
Environ. Pollut., 17: 39-51.

Ireland, M.P., 1979
Metal accumulation by the earthworms *Lumbricus rubellus, Dendrobaena veneta* and *Eiseniella tetraedra* living in heavy metal polluted sites.
Environ. Pollut., 19: 201-206.

Jackson, D.R., W.J. Selvidge and B.S. Ausmus, 1978
Behavior of heavy metals in forest microcosms: 1. Transport and distribution among components.
Water Air Soil Pollut., 10: 3-11.

Kelly, J.M., G.R. Parker and W.W. McFee, 1979
Heavy metal accumulation and growth of seedlings of five forest species as influenced by soil cadmium level.
J. Environ. Qual., 8: 361-364.

Killham, K. and M. Wainwright, 1981
Deciduous leaf litter and cellulose decomposition in soil exposed to heavy atmospheric pollution.
Environ. Pollut. (Ser. A), 26: 79-85.

Killingbeck, K.T., 1985
Autumnal resorption and accretion of trace metals in gallery forest trees.
Ecology, 66: 283-286.

Kirkham, M.B., 1981
Earthworms and soil physical conditions: Darwin to the present and future research needs. pp. 86-115, In M. Appelhof, ed., Workshop on the role of earthworms in the stabilization of organic residues, Vol. 1. Proceedings.
Beech Leaf Press, Kalamazoo, MI.

Laird, J.M. and M. Kroger, 1981
Earthworms.
CRC Critical Rev. Environ. Control, 11: 189-218.

Landin, M.C., 1978
Annotated tables of vegetation growing on dredged material throughout the United States.
Misc. Paper D-78-7, U.S. Army Engineer Waterways Experiment Station, Vicksburg, Miss., 155 pp.

Lea, R., W.C. Tierson, D.H. Bickelhaupt and A.L. Leaf, 1979
Stand treatment and sampling time of hardwood foliage. II Micro-element analysis.
Plant Soil, 51: 535-550.

Leavitt, S.W., R.D. Dueser and H.G. Goodell, 1979
Plant regulation of essential and non-essential heavy metals.
J. Appl. Ecol., 16: 203-212.

Little, P., 1973
A study of heavy metal contamination of leaf surfaces.
Environ. Pollut., 5: 159-172.

Ma, W.-C., 1982
The influence of soil properties and worm-related factors on the concentration of heavy metals in earthworms.
Pedobiologia, 24: 109-119.

Ma, W.-C., 1984
Sublethal toxic effects of copper on growth, reproduction and litter breakdown activity in the earthworm *Lumbricus rubellus*, with observations on the influence of temperature and soil pH.
Environ. Pollut. (Ser. A) 33: 207-219.

Maliszewska, W., S. Dec, H. Wierzbica and A. Wozniakowska, 1985
The influence of various heavy metal compounds on the development and activity of soil micro-organisms.
Environ. Pollut. (Ser. A) 37: 195-215.

Marquenie, J.M., 1984

Bioaccumulation of heavy metals and organic contaminants.
Third Interim Rept. No. R 84/126, MT-TNO, Delft, The Netherlands, 28 pp.

Marquenie, J.M. and J.W. Simmers, 1984

Bioavailability of heavy metals, PCB and PCA components to the earthworm (*Eisenia foetida*).
Proc. Internat. Conf. Environ. Contam. Toxicol., 10-13 July 1984, London.

Marquenie, J.M., J.W. Simmers and S.H. Kay, 1987

Preliminary assessment of bioaccumulation of metals and organic contaminants at the Times Beach confined disposal site, Buffalo, New York.
Misc. Paper EL-87-6, U.S. Army Engineer Waterways Experiment Station, Vicksburg, Miss.

Martin, M.H. and P.J. Coughtrey, 1982a

The use of vegetation for monitoring airborne heavy metal deposition. pp. 60-150, In M.H. Martin and P.J. Coughtrey, Biological monitoring of heavy metal pollution. Land and Air.
Applied Science Publishers, New York.

Martin, M.H. and P.J. Coughtrey, 1982b

Plants as monitors of soil contamination. pp. 151-220, In M.H. Martin and P.J. Coughtrey, Biological monitoring of heavy metal pollution. Land and Air
Applied Science Pulbishers, New York.

McCallan, S.E.A., R.H. Wellman and F. Wilcoxon, 1941

An analysis of factors causing variation in spore germination tests of fungicides. III Slope of toxicity curves, replicate tests, and fungi.
Contr. Boyce Thompson Inst. Plant Res., 12: 49-78.

McFarland, V.A., J.U. Clarke and A.B. Gibson, 1986

Changing concepts and improved methods for evaluating the importance of PCBs as dredged sediment contaminants.
Misc. Paper D-86-5, U.S. Army Engineer Waterways Experiment Station, Vicksburg, MS, 23 pp.

McKinney, J.D. and P. Singh, 1981
Structure-activity relationships in halogenated biphenyls: Unifying hypothesis for structural specificity.
Chem. Biol. Interactions 33: 271-283.

Meentemeyer, V., 1987
Macroclimate and lignin control of litter decomposition rates.
Ecology, 59: 465-472.

Melillo, J.M., J.S. Aber and J.F. Muratore, 1982
Nitrogen and lignin control of hardwood leaf litter decomposition dynamics.
Ecology, 63: 621-626.

Neuhauser, E.F., M.R. Malecki and R.C. Loehr, 1984
Growth and reproduction of the earthworm *Eisenia fetida* after exposure to sublethal concentrations of metals.
Pedobiologia, 27: 89-97.

Parker, G.R., W.W. McFee and J.M. Kelly, 1987
Metal distribution in forested ecosystems in urban and rural northwestern Indiana.
J. Environ. Qual., 7: 337-342.

Prins, E., 1982
Bioconcentrering van organochloorverbindingen in een voedselketen op de Vuilstortplaats in de Volgermeerpolder in Amsterdam.
RIN-Rapport 82-8, Rijksinstituut voor Natuurbeheer, Arnhem, Nederland, 48 pp.

Reaves, G.A. and M.L. Berrow, 1979
The lead content of the soils of the Wigtownshire area of Scotland.
J. Sci. Food Agric., 30: 1-7.

Reinecke, A.J. and J.R. Kriel, 1981
The influence of constant and diurnally fluctuating temperatures on the cocoon production, hatching time, and number of hatchlings of *Eisenia foetida* (Lumbricidae, Oligochaeta), pp. 166-177, In M. Appelhof ed., Workshop on the role of earthworms in the stabilization of organic residues, Vol. 1. Proceedings. Beech Leaf Press, Kalamazoo, MI.

Robers, B.L. and H.W. Dorough, 1984
Relative toxicities of chemicals to the earthworm *Eisenia foetida*.
Environ. Toxicol. Chem., 3: 67-78.

Russo, F. and E. Brennan, 1979
Phytotoxicity and distribution of cadmium in pin oak seedlings determined by mode of entry.
Forest Sci., 25: 328-332.

Satchell, J.E., 1983
Earthworm ecology in forest soils.
pp. 166-170, In J.E. Satchell, Earthworm Ecology, Cambridge.

Simmers, J.W., B.L. Folsom, Jr., C.R. Lee and D.J. Bates, 1981
Field survey of heavy metal uptake by naturally occurring saltwater and freshwater marsh plants.
Tech. Rept. EL-81-5, U.S. Army Engineer Waterways Experiment Station, Vicksburg, Miss., 161 pp.

Staaf, H., 1987
Foliage litter turnover and earthworm populations in three beech forests of contrasting soil and vegetation types.
Oecologia (Berlin), 72: 58-64.

Stachurski, A. and J. Zimka, 1982
Zinc cycling in forest ecosystems.
Pol. Ecol. Stud., 8: 343-359.

Stafford, E.A. and C.A. Edwards, 1985
Comparison of heavy metal uptake by *Eisenia foetida* with that of other common earthworms.
Final Tech. Rept., First Draft, Rothamsted Experimental Station, Harpenden, Herts., U.K. 78 pp.

Tyler, G., 1972
Heavy metals pollute nature, may reduce productivity.
Ambio, 1: 52-59.

Van Hook, R.I., 1974
Cadmium, lead, and zinc distributions between earthworms and soils: Potentials for biological accumulation.
Bull. Environ. Contam. Toxicol., 12: 509-512.

Wedding, J.B., R.W. Carlson, J.J. Stukel and F.A. Bazzaz, 1977
Aerosol deposition on plant leaves.
Water Air Soil Pollut., 7: 545-550.

Wheatley, G.A. and J.A. Hardman, 1968
Organochlorine insecticide residues in earthworms from arable soils.
J. Sci. Food Agric., 19: 219-223.

Yadav, D.V., P.K. Mittal, H.C. Agarwal and M.K.K. Pillai, 1981
Organochlorine insecticide residues in soil and earthworms in the Delhi area.
India, August-October, 1974, Pestic. Monit. J., 15: 80-85.

Zimka, J.R., A. Stachurski, A. Wasilewski, L. Grum and H. Mochnacka-Lawacz, 1981
The significance of species composition of forest stand in zinc transfer.
Pol. Ecol. Stud., 7: 137-144.